Faecal carriage of ESBL-producing *Escherichia coli* in a remote region of Niger

Running Title: Faecal carriage of ESBL-*E. coli* in Niger

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Summary

Objective

Whole genome sequencing (WGS) of extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-*E. coli*) in developing countries is lacking. Here we describe the population structure and molecular characteristics of ESBL-*E. coli* faecal isolates in rural Southern Niger.

Methods

Stools of 383 healthy participants were collected among which 92.4% were ESBL-*E. coli* carriers; 90 of these ESBL-*E. coli* containing stools (109 ESBL-*E. coli* isolates) were further analysed by WGS, using short- and long-reads.

Results

Most isolates belonged to the commensalism-adapted phylogroup A (83.5%), with high clonal diversity. The $bla_{\text{CTX-M-15}}$ gene was the major ESBL determinant (98.1%), chromosome-integrated in approximately 50% of cases, in multiple integration sites. When plasmid-borne, $bla_{\text{CTX-M-15}}$ was found in IncF (57.4%) and IncY plasmids (26.2%). Closely related plasmids were found in different genetic backgrounds. Genomic environment analysis of $bla_{\text{CTX-M-15}}$ in closely related strains argued for mobilisation between plasmids or from plasmid to chromosome.

Conclusions

Massive prevalence of community faecal carriage of CTX-M-15-producing *E. coli* was observed in a rural region of Niger due to the spread of highly diverse A phylogroup commensalism-adapted clones, with frequent chromosomal integration of $bla_{\text{CTX-M-15}}$. Plasmid spread was also observed. These data suggest a risk of sustainable implementation of ESBL in community faecal carriage.

Keywords: ESBL – *E. coli*, community, developing country, faecal carriage, chromosomal integration

46 Introduction

47 Extended-Spectrum β-Lactamase-producing (ESBL) Enterobacterales, and particularly *Escherichia*
48 *coli* (ESBL-*E. coli*) have spread worldwide and become endemic, especially in low-and middle-income
49 countries [1–5]. The diffusion of ESBL-*E. coli* is mainly the result of the epidemiological success of bla_{CTX-}
50 $_{M}$ genes in the 2000s, which are ESBL-encoding genes often carried by mobile genetic elements such
51 as plasmids. *E. coli* are commensals of the gut of vertebrates, including humans [6], and constitute the
52 main reservoir of resistance genes in Enterobacterales, leading to ESBL-*E. coli* dissemination in
53 communities [7]. *E. coli* are also opportunistic pathogens responsible for severe infections such as
54 bloodstream infections, urinary tract infections and in some cases diarrhea [8]. Infections with ESBL-*E.*
55 *coli*present a clinical challenge due to limited therapeutic options, which is especially crucial in low-
56 income countries [9]. Globally, ESBL-*E. coli* infections represent the biggest burden of bacterial
57 antimicrobial resistance (AMR) morbidity and mortality [10].

58 Prevalence of ESBL-*E. coli* faecal carriage in humans is a good indicator of the global burden of
59 antibacterial resistance in a given community [11]. In Africa, the few available studies showed a
60 prevalence of ESBL-*E. coli* community faecal carriage ranging from 15% to 92% (higher than in most
61 high income countries) depending on the country, the population studied and the year of stool
62 collection [2–5,12–14]. However, little is known concerning the molecular epidemiology and evolution of
63 resistance mechanisms in low-income countries [13,15]. Yet, this level of understanding is essential to
64 unravel the evolutionary mechanisms as well as the transmission mechanisms enabling the success
65 of ESBL-*E. coli* in community settings. It is crucial to determine specific strategies to prevent further
66 dissemination of these resistant bacteria. We previously reported that 92.4% of healthy individuals
67 carried ESBL-*E. coli* carried in their stools as part of a trial of ciprofloxacin prophylaxis for
68 meningococcal meningitis performed in a rural region of South Niger [14]. Faced with such a high
69 prevalence, we sought to describe the population structure of the strains and the molecular basis of
70 this resistance to understand the mechanisms of resistance spread.

71 **Patients and Methods**

72 **Study design**

73 This work was an ancillary study of a clinical trial on ciprofloxacin prophylaxis against bacterial
74 meningitis carried out between April 22, 2017 and May 18, 2017 during a meningococcal meningitis
75 outbreak in the Madarounfa district in rural southern Niger near the border with Nigeria [14].

76

77 **Population characteristics**

78 Briefly, a subset of 10 villages in the control arm and 10 villages randomized to receive village-wide
79 prophylaxis in the primary trial were randomly selected to participate in a sub-study examining faecal
80 carriage of ciprofloxacin-resistant and ESBL-producing *Enterobacterales*, at baseline and after
81 interventions [14]. In each village, 20 households were randomly selected from a census list. One
82 household member in each of the selected households was then selected using a simple lottery.
83 Villages were assigned an identification number and GPS coordinates were collected. Villages were
84 further described by the presence/absence of a market or a health center, as well as the distance to
85 the nearest paved road.

86 **Strain collection**

87 In the primary trial, stool samples of 383 individuals were collected at day 0 (prior to ciprofloxacin
88 administration in the intervention arm). Samples were collected at home by participants and
89 inoculated into Cary-Blair media (Copan, Brescia, Italy) by study staff. Of them, 99 faecal samples
90 were randomly selected and were sent to the Infection Antimicrobials Modelling and Evolution
91 laboratory (IAME, Université Paris Cité) for quality control in agar deep tubes [14]. In Paris, faeces were
92 plated on cefotaxime containing plates, and colonies of different morphologies were identified by
93 mass spectrometry (MALDI-TOF-MS Microflex Bruker Daltonics/ BioTyper™ version 2.0). When
94 colonies with different morphologies on the same plate were identified as *E. coli*, each isolate was
95 studied independently. Antimicrobial susceptibility testing was performed by the disk diffusion
96 method according to the EUCAST guidelines, and ESBL detection using the synergy test. Among the
97 99 samples, 90 samples from 90 subjects carrying at least one confirmed ESBL-*E. coli*, constitute the
98 material for the analyses performed in this work.
99

100 **Global genomic analysis**

101 For each *E. coli* isolate, DNA was extracted using NucleoMag DNA Bacteria for DNA purification
102 (Macherey Nagel, Hoerdt, France) and the whole genome was sequenced using Illumina Hiseq 2x100
103 bp after NextEra XT library preparation (Illumina, San Diego, CA, USA).
104 Sequences were analysed with a previously described in-house bioinformatic pipeline
105 [16](https://github.com/iame-researchCenter/Petanc). Briefly, *de novo* assembling was performed
106 using SPAdes 3.10.0 [17] and genomes were ordered with Ragout after genetic distance estimation
107 using Mash [18,19]. Genome typing was performed with *in silico* methods including phylogrouping [20],
108 multi-locus sequence typing (MLST) (according to the Warwick University and Pasteur Institute)[21],
109 serotyping [22], and *fim*H typing [23]. Plasmid replicons were determined using PlasmidFinder [24], and the
110 contig location (plasmid or chromosome) using Plascope [25]. Resistome was analyzed with Resfinder
111 v3.2 [26], and the virulome with a database based on Virulence Finder [27] and VFDB [28]. The main

112 virulence factors associated with extra-intestinal or intra-intestinal pathogenic *E. coli* (respectively
113 ExPEC and InPEC) were as in [29].
114
115 A core genome was determined using Roary v3.12 with default parameters [30], and a SNP distance
116 matrix was computed from the core genome (available in Supplementary Table 1). Then, a phylogeny
117 based on a maximum likelihood approach using a core genome alignment was performed using Iqtree
118 v1.6.12 [31]. Patristic distances obtained were used to design the phylogenetic tree with Itol (v6.3)[32].
119
120
121 **ESBL-encoded gene support determination**
122 For strains with ESBL-encoded genes embedded in chromosome, the integration sites of $bla_{\text{CTX-M-15}}$
123 was mapped on the linkage map of *E. coli* K-12[33], a A phylogroup strain ST10, assuming synteny wih
124 the studied isolates. Coordinates established by the completed sequence were expressed as 100
125 minutes for the entire circular map[33].
126
127 For strains with ESBL-encoded genes embedded in plasmids, an additional long-read sequencing was
128 performed. One representative isolate was selected for isolates with close genetic distances
129 according to Mash. The Nanopore sequencing (Oxford, UK) was performed using Ligation Sequencing
130 Kit (SQK-LSK109) with Native Barcoding Expansion 1-12 or 13-24, a FLO--min106 R9.4.1 flowcell, and
131 a MinION device Mk1B. The reads were filtered using Filtlong (v0.2.0)
132 (https://github.com/rrwick/Filtlong) to keep reads longer than 1000 nucleotides. The selected reads
133 were then assembled with the combination of minimap2 (v2.15) and miniasm (v0.3)[34]. The resulting
134 assembly was then polished through 2 rounds of minipolish (v0.1.0), a convenient wrapper for Racon
135 (V1.4.11)[35,36]. llumina and nanopore contigs were subsequently assembled using Unicycler[37].
136 Plasmids were compared with BRIG[38] when a scaffold was already available, or circlize R-package[39] in
137 absence of previously described reference. Plasmids with less than 1% difference in the nucleotide
138 sequences or ≤2 large insertions or deletions (indels) were considered as highly similar. Genome
139 annotation was performed using RAST[40].
140
141 **Ethics**
142 The parent study protocol, which included this sub-study, was reviewed and approved by the
143 National Consultative Ethics Committee of Niger (Ref: 003/2016/CCNE) and the Ethics Review Board
144 of Médecins Sans Frontières (Ref: 1603)[14]. The trial is registered at ClinicalTrials.gov (NCT02724046).
145 Written informed consent was obtained from individual participants.

146 **Results**

147 All raw data concerning antibiotic resistance and genome typing are available in Supplementary
148 Table 2.

149 **Population characteristics**

150 In this ancillary study, the median age of the 90 participants was 10.5 years [IQR 4-24.8], and the F:M
151 sex-ratio was 1.5. Only 3 subjects (3.3%) reported antibiotic exposure or hospitalization in the three
152 months prior to sample collection.

153 **ESBL-*E. coli* isolation and resistance to antibiotics**

154 A total of phenotypically distinct 109 ESBL-*E. coli* were recovered from the 90 samples. Seventy-two
155 participants carried only one ESBL-*E. coli,* 17 carried two distinct ESBL-*E. coli* and one participant
156 carried 3 distinct ESBL-*E. coli* by colony morphology (see above).

157 Among the 109 ESBL-*E. coli* isolates, 58 (53.2%) were resistant to nalidixic acid, 56 (51.4%) to
158 ciprofloxacin, 23 (21.1%) to gentamicin, 76 (69.7%) to cotrimoxazole, and 85 (78.0%) to tetracycline.
159 All were susceptible to amikacin and imipenem.

160 **Genomic diversity of ESBL-*E. coli***

161 The genomic diversity of ESBL-*E. coli* is shown in Table 1 and Figure 1. The ESBL-*E. coli* mainly
162 belonged to phylogroup A (91/109, 83.5%), phylogroup B1 (8/109, 7.3%) and phylogroup C (6/109,
163 5.5%). One single isolate was found in each of phylogroups D, F, B2 and clade I.

164 ST typing using Warwick University scheme highlighted 44 different STs. The principal STs identified
165 were ST46 (15/109, 13.8%), ST10 (10/109, 9.2%), ST6359 (7/109, 6.4%), ST617 (6/109, 5.5%), and
166 ST410 (6/109, 5.5%). *In silico* serotyping approaches identified 42 different serotypes, including 11
167 serotypes with unknown O antigens and new O antigens waiting for assignment for 24/109 (22%)
168 isolates. The main serotypes were O101:H9 (11/109, 10.1%), O101:H4 (10/109, 9.1%), O101:H10
169 (9/109, 8.3%), and O9:H9 (8/109, 7.3%). A total of 21 different *fimH* alleles were identified. For
170 34/109 (31.2%) isolates, no *fimH* gene was found. The main *fimH* alleles identified belonged to
171 *fimH54* (15/109, 13.8%), *fimH34* (11/109, 10.1%), *fimH24* (7/109, 6.4%), and *fimH41* (6/109, 5.5%).

172 **Clone definition and global epidemiology**

173 The definition of clone within the *E. coli* species is not trivial and has been refined at each
174 technological advance from serotype, MLST, *fimH* typing, to more recently whole genome
175 sequencing. The ST entity is heterogeneous in terms of divergence, and can encompass several
176 clones, as exemplified by the emerging ST131 with clades A, B and C having high levels of diversity[8].

177 Here, the clones were defined according the following strategy. First, isolates were grouped by their
178 haplogroup, defined as a combination of their sequence types (ST) according to the Warwick
179 University and Institute Pasteur schemes, their serotype (O:H), and their *fimH* allele. Second, the
180 distribution of SNPs among isolates was determined, and displayed a SNP cut-off around 120 where
181 few values were observed (Supplementary Figure 1). Third, SNP distances within and between
182 haplogroups were calculated and confronted to the cut-off. By using a cut-off at 120 SNPs, most
183 isolates belonging to a given haplogroup had a SNP distance below this cut-off (Supplementary Figure
184 1A). A diversity > 120 SNPs was found for few isolates belonging to a given haplogroup: clones 4/5
185 (683 SNPs), clones 12/14 (2565 SNPs) and clones 34/35 (173 SNPs)(Figure 1). Conversely, the clone
186 36 was associated with two different haplogroups, due to differences in ST assignation. It should be
187 noted that strain ST10479 is a single locus variant of strain ST46, highlighting a high degree of
188 similarity. Similar results have been found with patristic distances (Supplementary Figure 1B). We
189 thus defined the clone as encompassing isolates with less than 120 SNPs on their core genomes.
190 Based on this definition, 57 distinct clones were identified among the 109 isolates. Of 109 isolates, 74
191 (67.9%) belonged to clones represented by two or more isolates. Isolates mainly belonged to clones
192 36 (10/109, 9.2%), 37 (5/109, 4.6%) and 26 (5/109, 4.6%). Of note, *E. coli* strains with different
193 morphologies from the same individual were always genetically distinct (Table1, Figure 1).
194 In summary, despite belonging almost uniquely to phylogroup A, a huge genomic diversity of clones
195 was observed among the ESBL-*E. coli* isolates.

196 **ESBL-encoding genes and genomic location**

197 The ESBL phenotype was conferred by the $bla_{\text{CTX-M-15}}$ gene for 107/109 (98.1%) isolates. The $bla_{\text{CTX-M-15}}$
198 gene was located on the chromosome for 48/107 (44.9%) isolates and on a plasmid for 59/107
199 (55.1%) isolates. The remaining two isolates had the ESBL phenotype conferred by $bla_{\text{SHV-2a}}$ (1 isolate)
200 and $bla_{\text{CTX-M-55}}$ genes (1 isolate), and were both located on a plasmid.

201 The 48 isolates with a chromosomal $bla_{\text{CTXM-15}}$ gene were phylogenetically diverse, belonging to 23
202 clones. We identified 19 different chromosome integration sites (Figure 1 and Supplementary Table
203 3). For most isolates, a given chromosome integration site was associated with a given clone (Figure
204 1). For a single clone (clone 36), we observed two different integration sites (Supplementary Table 3).
205 Altogether, these data indicate multiple integration events in diverse genetic backgrounds. The
206 distribution of integration sites on the chromosome of *E. coli* K-12 (phylogroup A) has been mapped,
207 assuming global synteny between studied isolates and K-12 (Figure 2). Integration sites have been
208 found in different chromosomal regions, but are not evenly distributed across the genome. Most of

209 the integration sites are within the Ter macrodomain whereas no integration has been identified in
210 the Ori macrodomain [41]

211 For isolates with ESBL-encoding gene located on a plasmid, 10 different replicon types were
212 identified. The plasmids with *bla*$_{SHV-2a}$ and *bla*$_{CTX-M-55}$ genes belonged to IncN and IncX1 incompatibility
213 groups, respectively. The plasmids harbouring *bla*$_{CTX-M-15}$ belonged mainly to IncF (IncFIA (5/107),
214 IncFIB (16/107), IncFIB (H89 phagemid plasmid) (6/107), IncFIC(FII) (8/107)) and IncY (16/107) and, to
215 a lesser extent, by IncI1 (6/107) incompatibility groups (Table 1, Figure 3).

216 To better understand the relationships between the clones and the plasmids, we studied the isolates
217 for which the plasmids showed a strong similarity (see above). In six cases, we found highly similar
218 plasmids in different genetic backgrounds, whereas 20 plasmids were strictly associated with a single
219 genetic background (for plasmid alignments, see Supplementary Figure 2). For incFIA plasmids, we
220 identified similar plasmids, with insertion of 26.7 kbp in the plasmid hosted by clone 8 compared to
221 that of clone 9. For IncFIB plasmids, we identified similar plasmids in clones 28 and 50, with insertion
222 of 26.7 kbp in the plasmid hosted by clone 28, and an insertion of 8.8 kbp in the plasmid hosted by
223 clone 50. Moreover, the plasmid hosted by clone 29 presented a 27.9 kbp insertion compared to that
224 of clone 45. For IncI1 plasmids, plasmids hosted by clones 16 and 25 presented more than 99%
225 identity, and the ones hosted by the clones 17, 30, and 56 presented two indels of 2.14 and 1.34 kbp.
226 For IncY plasmids, we identified similar plasmids, with insertion of 17 kbp in the plasmid hosted by
227 clone 32 compared to that of clone 37. Altogether, these data suggest plasmid transmission between
228 clones.

229 **Focus on evolutionary scenarios of *bla*$_{CTX-M-15}$ gene spread in closely related clones**

230 If our analyses suggest both clonal and plasmid spreads, we wanted to go further in the unravelling
231 of putative evolutionary scenarios leading to the diffusion of *bla*$_{CTX-M-15}$ gene in closely related
232 isolates. Here we focus on two clusters: one with clones 1 to 7, and a second with clones 36 and 37.

233 *Focus on clones 1 to 7*

234 For isolates with *bla*$_{CTX-M-15}$ gene embedded in the integration site "A" (clones 1 to 5 and 7), we
235 assume that *bla*$_{CTX-M-15}$ gene acquisition would have followed the most parsimonious scenario:
236 isolates with chromosomal *bla*$_{CTX-M-15}$ gene have diversified after a single event of integration in a
237 common ancestor. This assumption is based on the fact that the insertion site was the same for all
238 isolates with chromosomal *bla*$_{CTX-M-15}$ and that IS*EcpI* upstream *bla*$_{CTX-M-15}$ is largely deleted (data not
239 shown). In addition, in the closely-related clone 6, *bla*$_{CTX-M-15}$ gene was acquired through an IncFIA
240 plasmid.

241

242 *Focus on clones 36* and *37*

243 Conversely, in clone 36/ST46, $bla_{CTX-M-15}$ is hosted in two different chromosome integration sites, J (six
244 isolates) and K (two isolates), suggesting two independent integration events in the same clone. In
245 addition, in the closely-related clones 36/ST10479 and clone 37, $bla_{CTX-M-15}$ gene was acquired by the
246 IncFIB and IncY plasmids, respectively.

247 Analysis of synteny in the regions flanking the $bla_{CTX-M-15}$ gene (Figure 4) displays highly conserved
248 genetic environment, suggesting transfer between plasmid and chromosome and/or between
249 plasmids.

250 **Virulence associated genes**

251 We focused on the main virulence factors associated with extra-intestinal or intra-intestinal
252 pathogenic *E. coli* (respectively ExPEC and InPEC)(19). The average number of ExPEC virulence genes
253 was low (1.24), the most prevalent being *fuyA* and *irp2* (25/109, 22.9%) (Supplementary Table 2).
254 These two genes encode the yersiniabactin siderophore and its transporter, and are known to be
255 part of the high pathogenicity island (HPI)[42]. For phylogroup A isolates, these two genes were
256 unevenly distributed among the strains, and were mostly found in a cluster of clones (clones 1 to 6,
257 and clone 8). In the phylogroup C, most isolates (4/6) harboured the adhesin encoding *pap* genes in
258 addition to *fuyA* and *irp2* genes. One enterotoxinogenic *E. coli* (ETEC) (*eltB* positive) from clade I and
259 two enteropathogenic *E. coli* (EPEC) from B1 and B2 phylogroups were identified, the latter belonging
260 to the ST2346 and being a typical EPEC (*eae* and *bfp* positive)[43].

261 **Geographical origin of ESBL-*E. coli* carriers**

262 The geographical distribution of the villages of the carriers of the 109 ESBL-*E. coli* is represented in
263 Figure 5.
264 Most isolates belonging to the same clone were not limited to a given geographical area, but were
265 widely spread across the different villages (such as clones 1, 8, 15, 20, 36, 37, 40, 41, and 42).
266 Nevertheless, few clones, had a local spread limited to 1 or 2 close villages (such as clones 3, 18, 19,
267 24, and 26). Last, some clones widely spread in the eastern area, in villages connected by a paved
268 road and which depend on the same healthcare center (clones 11, 30, 31, 32, 38, 51, and 53).
269 The isolates 3_13 (belonging to clone 50) and the isolates 3_12 and 3_27 (belonging to clone 28)
270 harbour plasmids with strong similarity, and were identified in the same village (village 3), suggesting
271 a putative plasmid transfer between isolates with different genetic backgrounds (Figure 1 and Figure
272 4).

273 In sum, we did not find any correlation between geographical localization of the subjects and the
274 epidemiological genomic data of ESBL-*E. coli*, which suggests a large spread of the most frequent
275 clones.

276 **Discussion**

277 After finding an unexpectedly high prevalence of ESBL-*E. coli* faecal carriage in healthy subjects
278 (>90%) in rural South Niger [14], we analysed the population structure, molecular support of resistance
279 and mode of spread of the resistance in these commensal strains, using state-of-the-art sequencing
280 techniques with both Illumina WGS and an additional long-read sequencing by Nanopore to
281 specifically study strains with ESBL-encoded genes embedded in plasmids. Previous studies of ESBL
282 carriage in African settings has varied by the region, the community and the age of the subjects [2–
283 5,12,13]. Reported prevalence in Africa has been high, ranging from 46 % in district hospital patients in
284 South Africa in 2018 [3], 51% in hospitalized patients and 38% in healthy healthcare workers in Chad in
285 2019 [13], and 59% in healthy children in the community in the Central African Republic [12]. When
286 resistance mechanisms and phylogenetic groups were described, $bla_{CTX-M-15}$ was the most commonly
287 encountered gene encoding for resistance to β-lactams (ranging from 75% to 90% of cases), and the
288 clonal group ST131 was predominant among phylogroup B2 *E. coli* [12,13,15,44].

289 The main features of our work are the following. First, most ESBL-*E. coli* belonged to phylogroup A
290 (83.4%), but with a high diversity in terms of STs, serotypes, *fimH* alleles and finally clones, even
291 though some clones were overrepresented. *E. coli* strains belonging to phylogroup A are known to be
292 adapted to human gut commensalism lifestyle [6] and have been described as predominant in low-and
293 middle-income countries [45]. As expected, these strains carry few virulence genes, with the exception
294 of a cluster of clones (clones 1 to 6, and clone 8), which have accumulated many virulence genes,
295 especially iron capture systems such as yersiniabactin (*fyuA, irp2*), borne on the HPI (Supplementary
296 Table 2). ST410 (phylogroup C), an emerging high-risk clone [46], is also well represented, with strains
297 harbouring the *pap* adhesin encoding genes in addition to the HPI. Both gene clusters have been
298 associated with ESBL-*E. coli* strains with a longer duration of faecal carriage in travellers returning
299 from developing countries [47]. On the other hand, B2 phylogroup was represented by a single isolate,
300 a typical EPEC belonging to the EPEC9 cluster [43,48,49]. This is in contrast to what has been observed in
301 Western countries, where the proportion of B2 strains among commensal *E. coli* has dramatically
302 increased in the last four decades, probably under the pressure of several factors such as changes in
303 dietary habits and improved hygiene [6,50]. Of note, no ST131-*E. coli* isolate (also a B2) was found here,
304 despite the clone's global epidemiological success [12,13,51]. The pronounced diversity among A strains
305 and the low prevalence of B2 strains supports the hypothesis that the strains described here are
306 almost individual-specific, and aligns with the expected composition of commensal *E. coli* strains

307 from humans living in developing countries. Along the same line, a recent study performed in healthy
308 pregnant women in Madagascar showed a high prevalence of ESBL-*E. coli* (34%), composed mostly of
309 isolates belonging to various phylogroup A clones (72%)[52].

310 Second, we found that the ESBL phenotype was mainly associated with the $bla_{CTX-M-15}$ gene (98%),
311 which was embedded in the chromosome in approximately 50% of cases, at different integration
312 sites. The spread of the $bla_{CTX-M-15}$ gene has been well-described in the last decades, even though
313 other bla_{CTX-M} alleles such as $bla_{CTX-M-14}$ or $bla_{CTX-M-27}$ have had some epidemiological success [2]. As with
314 most AMR genes, the spread of bla_{CTX-M} genes has been associated with plasmid dissemination,
315 particularly the pC15-1a plasmid [53]. However, chromosomal locations have increasingly been
316 described. Chromosome-located bla_{CTX-M} genes were found in 4.8% of ESBL-*E. coli* isolates in a
317 veterinary collection from Germany, the Netherlands, and the UK [54]. This rate increases to 8.3% and
318 14.6%, respectively, in clinical isolates recovered from hospitals in Zambia [55] and China [56]. In the
319 study from Madagascar cited above [52], the bla_{CTX-M} gene was located on the chromosome in 31% of
320 strains. The highest incidence of chromosomal bla_{CTX-M} gene described so far was observed in Japan,
321 where $bla_{CTX-M-14}$ was found on the chromosome in 45.9% of cases, and in 16.2% both on
322 chromosome and plasmid in community commensal strains [57]. The location of bla_{CTX-M} genes on both
323 chromosome and plasmid probably reflects the ability of their genetic environment to mobilize these
324 resistance genes. In their model, Hamamoto *et al.* display high rates of $bla_{CTX-M-14}$ mobilization, and
325 integration in various sites [58], as was observed here with our isolates. Whether a chromosomal or
326 plasmid location confers a selective advantage for the resistance genes is a matter of debate. In a
327 mathematical model, Lethtinen *et al.* suggested that plasmid location could be preferred under
328 selective pressure [59], suggesting that a chromosomal location is more adapted to a lower levels of
329 selective pressure, as would be the case in community carriage. This hypothesis is also supported by
330 the fitness cost associated with plasmid-carried resistance genes [60] and by the conservation of
331 vertical transfer of chromosome-located genes. These assumptions remain challenged by other
332 authors, as compensatory mutations alleviating plasmid fitness costs and high plasmid-chromosome
333 beneficial interactions have also been described [60]. From an epidemiological point-of-view, a recent
334 work suggested that chromosome integration of $bla_{CTX-M-15}$ in ST131 isolates could have contributed
335 to the clonal expansion of a rare C2 lineage [61].

336 Third, for isolates with plasmid-located $bla_{CTX-M-15}$, we showed a predominance of IncF plasmids,
337 known to be well adapted to *Enterobacterales*, followed by IncY plasmids, also reported in
338 Madagascar. Interestingly, a high similarity was found for some plasmids in different genetic
339 backgrounds, suggesting both isolate and plasmid diffusion. This observation here in a human
340 community is in line with a previous study of temporal intestinal carriage of ESBL-*E. coli* in calf farms

341 [62]. Additionally, we found a similarity between IncFIb plasmid backbones and pCFSAN061762, a
342 plasmid described in Egypt in an isolate of *E. coli* ST38 (data not shown).

343 Fourth, careful genetic analysis of the $bla_{CTX\text{-}M\text{-}15}$ gene in the ST46 and ST46 single locus variant clones
344 (Figure 4) argues for mobilisation of the ESBL gene with variable numbers of flanking genes between
345 plasmids and from plasmids to chromosome. This process has been recently suggested in A
346 phylogroup strains isolated during a "One Health" study on Reunion island [63].

347 Finally, we did not find any correlation between geographical localization of the subjects (the villages
348 they live in or their usual social interactions, such as marketplaces) and the epidemiological genomic
349 data of ESBL-*E. coli*, which suggests a large spread of the most frequent clones. These results may
350 unveil an underestimated mix of populations in this area, or contamination by a common source of
351 food or water that could not be captured by mapping health centers, distance to an asphalt road or
352 marketplaces, as represented in Figure 4.

353 Our work has some limitations: (i) only a subset of stool samples from the overall study was analysed,
354 potentially introducing biases, yet the random selection should have reduced this risk; (ii) only the
355 predominant cefotaxime-resistant *E. coli* isolates present in the faeces were studied, which may not
356 represent the diversity of *E. coli* isolates in the gut, but does represent the quantitatively
357 predominant *E. coli* isolates and therefore the ones that constitute the biggest threat in terms of
358 infectious risks and dissemination [64]; (iii) the search of an ESBL-*E. coli* reservoir in other
359 environments, such as the food chain (poultry/livestock), water or sediments has not been planned,
360 impeding the understanding of the routes of dissemination in a "One Health" approach [11].

361 Despite these limitations, this epidemiologic study in a remote region of Niger is unique and shows
362 the unprecedented dissemination of ESBL-*E. coli* in the gut of a geographically localized human
363 population. It suggests the widespread dissemination of multiple resistant clones, possibly fostered
364 by underestimated inadequate antibiotic treatments and multiple population exchanges that have
365 probably lasted for years already. From a molecular point of view, this work highlights the diversity of
366 pathways followed by $bla_{CTX\text{-}M\text{-}15}$. The high frequency of chromosomal $bla_{CTX\text{-}M\text{-}15}$ in isolates of *E. coli*
367 belonging to the commensal phylogroup A is of particular concern because it could be associated
368 with their sustainable implementation in the community. Understanding the evolutionary forces that
369 select for such a high prevalence of ESBL-*E. coli* should be major objective.

370 Altogether, antibiotic resistance in commensal *E. coli* in this remote part of Africa appears to have all
371 the characteristics of long-term adaptation and all the tools for further dissemination. This is
372 especially worrying as commensal *E. coli* are responsible for clinical infections and antimicrobial
373 resistant *E. coli* infections represent the biggest threat in terms of AMR globally [10]. Noteworthy,

374 community-acquired bacteremia in children hospitalized for severe acute malnutrition was recently
375 followed in an intensive nutritional rehabilitation center in the same rural district. Approximately
376 two-thirds of the *E. coli* and *K. pneumoniae* strains (16/26, 61.5%) were ESBL producers, reinforcing
377 the link between the widespread carriage of ESBL-producing *E. coli* strains and the risk of severe
378 infection from these same microorganisms [65]. Infections with these strains will be increasingly
379 difficult to treat, with the need for broader spectrum antibiotics, which in turn may select more
380 resistant strains such as carbapenemase producers. This work may just be exposing the tip of the
381 iceberg of an AMR epidemiological catastrophe well on its way.

382

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Declaration of interest statement

None

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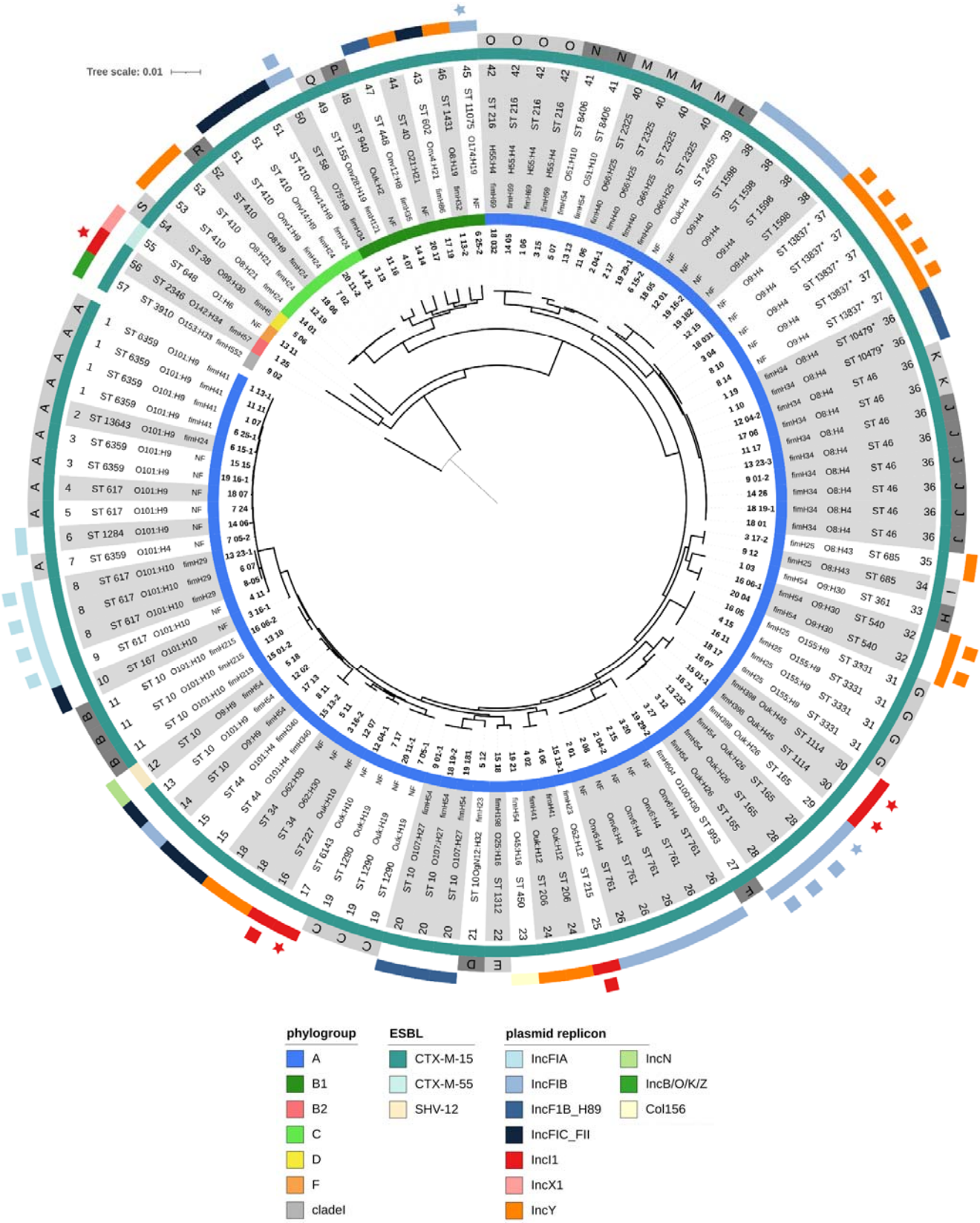


Figure 1. Core genome SNP-based phylogenetic tree of 109 ESBL-*E. coli*. The tree is rooted on *E. coli* clade I (isolate 9_02 (clone 47)). From center to periphery, the different layers correspond to the isolate name, the phylogenetic group, the *fimH* allele, the serotype, the sequence type (according the Warwick University scheme), the clone number, the ESBL-type, the chromosome integration site, and the CTX-M carrying plasmid replicon type. In the layer dedicated to ST type, ST10479 and ST13837 (clones 36 and 37) are single locus variant of ST46 and annotated with a star. Squares and stars

are used alternatively to separate isolates. Chromosome integration sites (A to S) are described in Supplementary Table 3.

Figure 2. Location of integration sites of *bla*$_{CTX-M-15}$ on the linkage map of *E. coli* K-12. Coordinates established by the completed sequence are expressed as 100 minutes for the entire circular map. The height of each bar is proportional to the number of isolates with a given integration site.

Macrodomains are indicated by different colors : Left (13'-26') in yellow, Ter (26'-47') in green, Right (47'-62') in grey and Ori (81'-1') in blue, as described by Valens *et al.* [41].

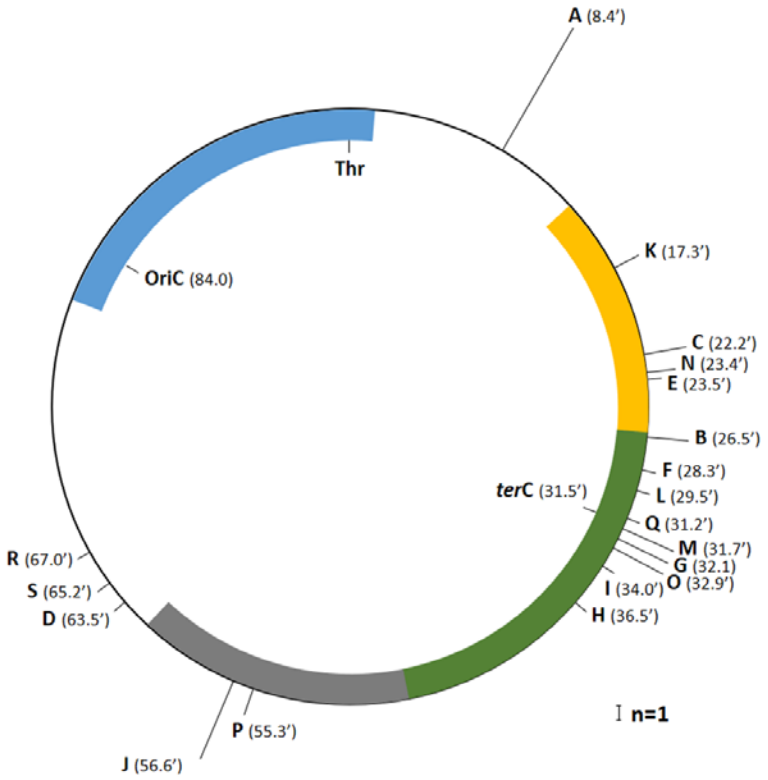


Figure 3. Prevalence of plasmid replicons harboring ESBL-encoding genes.

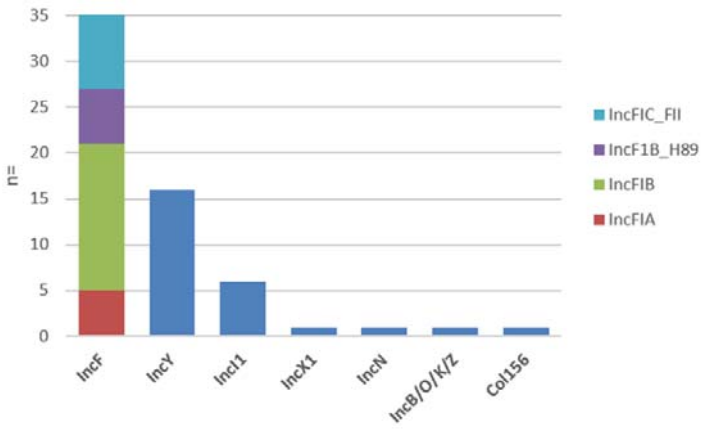
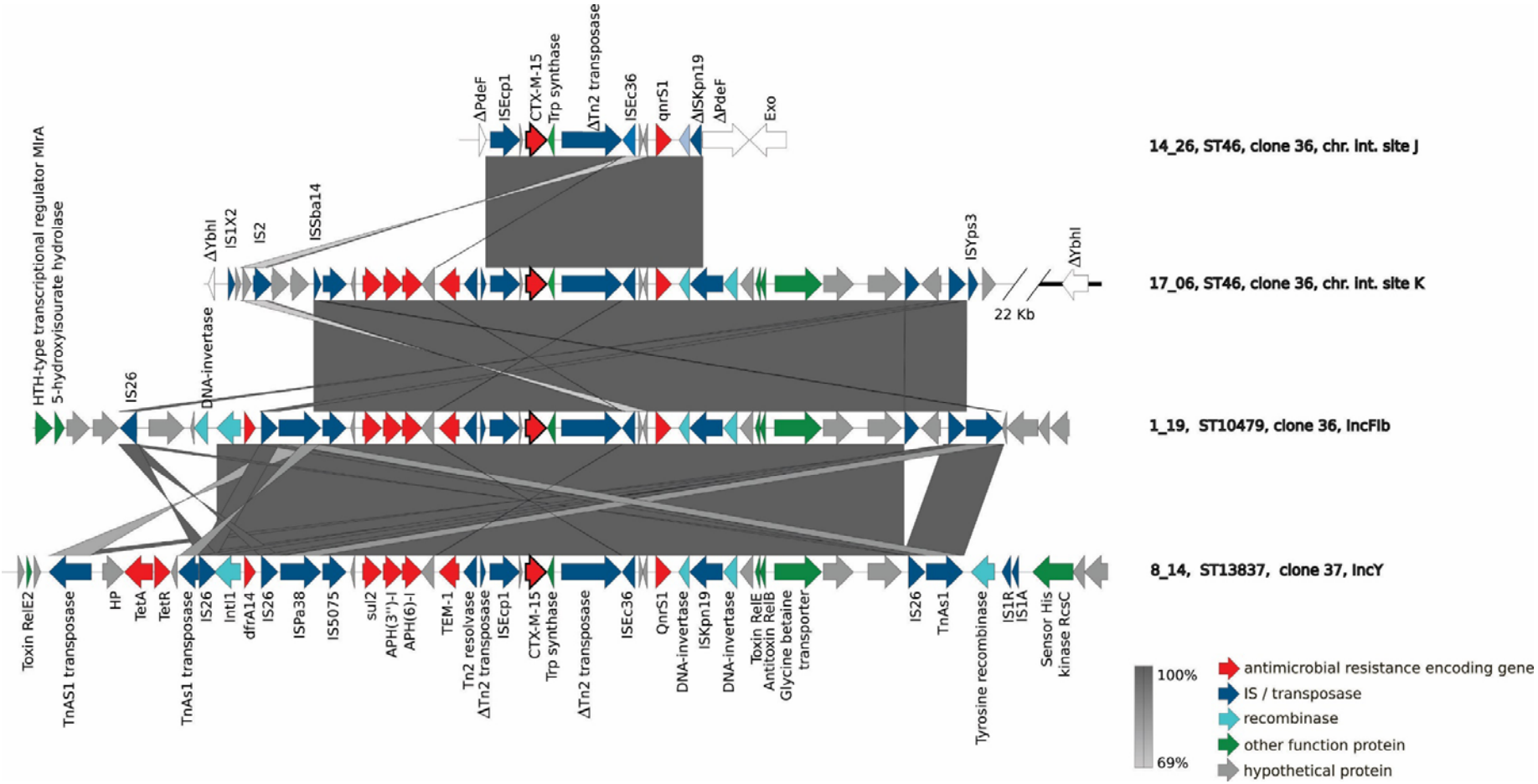
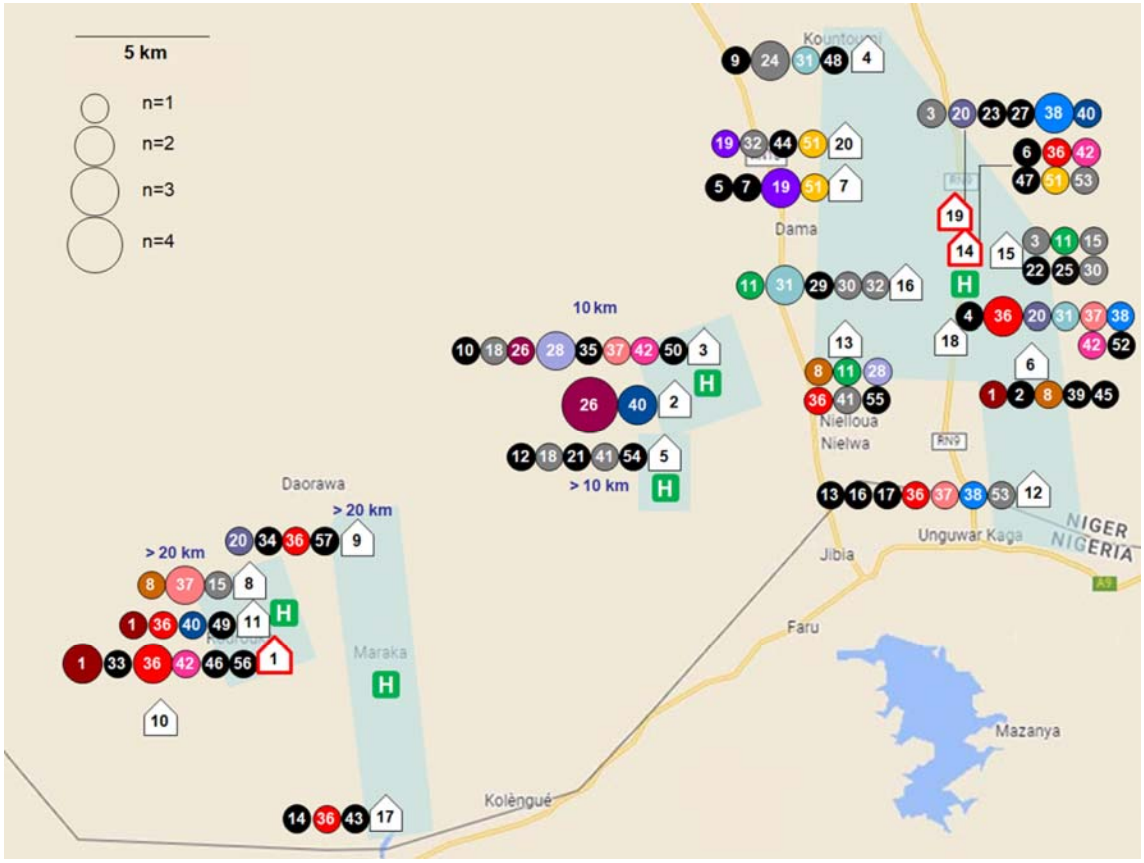


Figure 4. Genetic environments of CTX-M-15 encoding genes for four isolates representative of different genetic patterns of ST46 and ST46 single locus variants



For the four isolates, the number of the isolate, the single variant locus of ST46, the clone number, and the chromosome integration site or plasmid type are indicated on the right of the figure.

Figure 2. Geographical location of different clones of ESBL-*E. coli*



House icons represent the locations of the villages. No isolates were recovered from village 10. Villages with a market are bordered in red. Healthcare centres are indicated by a green box with an H. Villages belonging to the same healthcare district appear in the light blue shapes. Distance to the nearest paved road are indicated in blue. For each village, circles represent clones whose area is proportional to the number of isolates. Circles coloured with different colours correspond to clones represented by more than two isolates, those in grey and black to clones represented by two or one isolate, respectively.

Table 1. Characteristics of ESBL-*E. coli* according to genome typing and geographical location

Phylogroup (n)	ST[a] (n)	Serotype[b] (n)	*fimH* allele[c] (n)	Clone[d] (n)	ESBL-typing	Genomic location[e]	Village code[f] (n)
A (91)	ST6359 (7)	O101:H9 (6)	fimH41 (4)	1 (4)	CTX-M-15	chr	1(2), 6, 11
			NF (2)	3 (2)	CTX-M-15	chr	15, 19
		O101:H4 (1)	NF (1)	7 (1)	CTX-M-15	chr	7
	ST617 (6)	O101:H10 (4)	fimH29 (3)	8 (3)	CTX-M-15	IncFIA	6, 8, 13
			NF (1)	9 (1)	CTX-M-15	IncFIA	4
		O101:H9 (2)	NF (2)	4 (1)	CTX-M-15	chr	18
				5 (1)	CTX-M-15	chr	7
	ST13643 (1)	O101:H9 (1)	fimH24 (1)	2 (1)	CTX-M-15	chr	6
	ST1284 (1)	O101:H9 (1)	NF (1)	6 (1)	CTX-M-15	IncFIA	14
	ST167 (1)	O101:H10 (1)	NF (1)	10 (1)	CTX-M-15	IncFIC_FII	3
	ST10 (3)	O101:H10 (3)	fimH215 (3)	11 (3)	CTX-M-15	chr	13, 15, 16
	ST10 (1)	O9:H9 (1)	fimH54 (1)	12 (1)	SHV-12	IncN	5
	ST10 (1)	O101:H9 (1)	fimH54 (1)	13 (1)	CTX-M-15	IncFIC_FII	12
	ST10 (1)	O9:H9 (1)	fimH54 (1)	14 (1)	CTX-M-15	IncFIB	17
	ST44 (2)	O101:H4 (2)	fimH340 (2)	15 (2)	CTX-M-15	IncFIC_FII	8, 15
	ST227 (1)	Ouk:H10 (1)	NF (1)	16 (1)	CTX-M-15	IncI1	12
	ST6143 (1)	Ouk:H10 (1)	NF (1)	17 (1)	CTX-M-15	IncI1	12
	ST34 (2)	O62:H30 (2)	NF (2)	18 (2)	CTX-M-15	IncY	3, 5
	ST1290 (3)	Ouk:H19 (3)	NF (3)	19 (3)	CTX-M-15	chr	7(2), 20
	ST10 (3)	O107:H27 (3)	fimH54 (3)	20 (3)	CTX-M-15	IncFIB_H89	9, 18, 19
	ST10 (1)	OgN12:H32 (1)	fimH23 (1)	21 (1)	CTX-M-15	chr	5
	ST1312 (1)	O25:H16 (1)	fimH198 (1)	22 (1)	CTX-M-15	chr	15
	ST450 (1)	O45:H16 (1)	fimH54 (1)	23 (1)	CTX-M-15	Col156	19
	ST206 (2)	Ouk:H12 (2)	fimH41 (2)	24 (2)	CTX-M-15	IncY	4(2)
	ST215 (1)	O62:H12 (1)	fimH23 (1)	25 (1)	CTX-M-15	IncI1	15
	ST761 (5)	Onv6:H4 (5)	NF (5)	26 (5)	CTX-M-15	IncFIB	2(4), 3
	ST993 (1)	O100:H30 (1)	fimH604 (1)	27 (1)	CTX-M-15	chr	19
	ST165 (4)	Ouk:H26 (4)	fimH54 (3)	28 (3)	CTX-M-15	IncFIB	6, 8, 13
			fimH398 (1)	29 (1)	CTX-M-15	IncFIB	16
	ST1114 (2)	Ouk:H45 (2)	fimH398 (2)	30 (2)	CTX-M-15	IncI1	15, 16
	ST3331 (4)	O155:H9 (4)	fimH25 (4)	31 (4)	CTX-M-15	chr	4, 16(2), 18
	ST540 (2)	O9:H30 (2)	fimH54 (2)	32 (2)	CTX-M-15	IncY	16, 20
	ST361 (1)	O9:H30 (1)	fimH54 (1)	33 (1)	CTX-M-15	chr	1
	ST685 (2)	O8:H43 (2)	fimH25 (2)	34 (1)	CTX-M-15	chr	9
				35 (1)	CTX-M-15	IncY	3
	ST46 (8)	O8:H4 (8)	fimH34 (8)	36 (8)	CTX-M-15	chr	9, 11, 12, 13, 14, 17, 18(2)
	ST10479*(2)	O8:H4 (2)	fimH34 (2)	36 (2)	CTX-M-15	chr	1 (2)
	ST13837*(5)	O9:H4 (5)	NF (5)	37 (5)	CTX-M-15	IncY	3, 8(2), 12, 18
	ST1598 (4)	O9:H4 (4)	NF (4)	38 (4)	CTX-M-15	IncFIB	12, 18, 19(2)
	ST2450 (1)	Ouk:H4 (1)	NF (1)	39 (1)	CTX-M-15	chr	6
	ST2325 (4)	O66:H25 (4)	fimH40 (4)	40 (4)	CTX-M-15	chr	2(2), 11, 19
	ST8406 (2)	O51:H10 (2)	fimH54 (2)	41 (2)	CTX-M-15	chr	5, 13
	ST216 (4)	H55:H4 (4)	fimH69 (4)	42 (4)	CTX-M-15	chr	1, 3, 14, 18
B1 (8)	ST602 (1)	Onv4:H21 (1)	fimH86 (1)	43 (1)	CTX-M-15	IncFIC_FII	17
	ST40 (1)	O21:H21 (1)	NF (1)	44 (1)	CTX-M-15	IncY	20
	ST11075 (1)	O174:H19 (1)	NF (1)	45 (1)	CTX-M-15	IncFIB	6
	ST1431 (1)	O8:H19 (1)	fimH32 (1)	46 (1)	CTX-M-15	IncY	1
	ST448 (1)	Onv12:H8 (1)	fimH35 (1)	47 (1)	CTX-M-15	IncFIB_H89	14
	ST940 (1)	Ouk:H2 (1)	NF (1)	48 (1)	CTX-M-15	chr	4
	ST155 (1)	Onv28:H19 (1)	fimH121 (1)	49 (1)	CTX-M-15	chr	11
	ST58 (1)	O75:H9 (1)	fimH34 (1)	50 (1)	CTX-M-15	IncFIB	3
C (6)	ST410 (3)	Onv14:H9 (2)	fimH24 (2)	51 (2)	CTX-M-15	IncFIC_FII	14, 20
		Onv1:H9 (1)	fimH24 (1)	51 (1)	CTX-M-15	IncFIC_FII	7

	ST410 (1)	O8:H9 (1)	fimH24 (1)	52 (1)	CTX-M-15	chr	18
	ST410 (2)	O8:H21 (2)	fimH24 (2)	53 (2)	CTX-M-15	IncY	12, 14
D (1)	ST38 (1)	O99:H30 (1)	fimH5 (1)	54 (1)	CTX-M-15	chr	5
F (1)	ST648 (1)	O1:H6 (1)	NF (1)	55 (1)	CTX-M-55	IncX1	13
B2 (1)	ST2346 (1)	O142:H34 (1)	fimH57 (1)	56 (1)	CTX-M-15	IncI1	1
cladeI (1)	ST3910 (1)	O153vr2:H33 (1)	fimH552 (1)	57 (1)	CTX-M-15	IncBOKZ	9

[a]STs are assignated according to the Warwick scheme. STNF: ST not found. ST10479* and ST13837* correspond to single locus variants of ST46 in *icd*8 and *fumC*8 alleles, respectively.

[b]Ouk : unknown O antigen, Onv : new O antigen waiting for assignment.

[c]NF : not found.

[d]Isolates that belong to the same clone are designated by a dedicated number.

[e]plas : plasmid, chr : chromosome. When located on the plasmid, the incompatibility group is noticed

[f]villages are designated by a number, and are mapped in Figure 5.