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1 **Dissemination of IncI plasmid encoding *bla*<sub>CTX-M-1</sub> is not hampered by its fitness cost in**  
2 **the pig's gut.**

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16

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21

22 ABSTRACT

23 Multiresistance plasmids belonging to the IncI incompatibility group have become one of the  
24 most pervasive plasmid types in extended-spectrum beta-lactamase producing *Escherichia coli*  
25 of animal origin. The extent of the burden imposed on the bacterial cell by these plasmids seems  
26 to contribute to the emergence of “epidemic” plasmids. However, *in vivo* data in the natural  
27 environment of the strain are scarce. Here, we investigated the cost of a *bla*<sub>CTX-M-1</sub>-IncI1  
28 epidemic plasmid in a commensal *E. coli* animal strain, UB12-RC, before and after oral  
29 inoculation of fifteen 6- to 8-week-old specific pathogen-free pigs. Growth rate in rich medium  
30 was determined on (i) UB12-RC and derivatives, with or without plasmid, *in vivo* and/or *in*  
31 *vitro* evolved, and (ii) strains that acquired the plasmid in the gut during the experiment.  
32 Although *bla*<sub>CTX-M-1</sub>-IncI1 plasmid imposed no measurable burden on the recipient strain after  
33 conjugation and during the longitudinal carriage in the pig’s gut, we observed a significant  
34 difference in the bacterial growth rate between IncI1 plasmid-carrying and plasmid-free isolates  
35 collected during *in vivo* carriage. Only a few mutations on the chromosome of the UB12-RC  
36 derivatives were detected by whole-genome sequencing. RNA-Seq analysis of a selected set of  
37 these strains showed that transcriptional responses to the *bla*<sub>CTX-M-1</sub>-IncI1 acquisition were  
38 limited, affecting metabolism, stress response, and motility functions. Our data suggest that the  
39 effect of IncI plasmid on host cells is limited, fitness cost being insufficient to act as a barrier  
40 to IncI plasmid spread among natural population of *E. coli* in the gut niche.

41

42 INTRODUCTION

43           Since the 2000s, a global epidemic of Extended Spectrum Beta-Lactamase (ESBL)-  
44 producing *Enterobacteriales* has emerged, linked to the dissemination of successful *bla*<sub>CTX-M</sub>-  
45 carrying clones (1). The CTX-M enzymes are mostly plasmid encoded and *E. coli* is the major  
46 host (2).

47           Among major multiresistant plasmids in *Enterobacteriales*, IncF, IncI, IncC, IncN, and  
48 IncL plasmid families are the most commonly reported. The I-complex plasmids contain  
49 incompatibility groups I, K, B, and Z. IncI, or MOB<sub>P</sub> according to relaxase typing, is a group  
50 of low copy-number, narrow-host-range, conjugative plasmids, which vary in size from 50 to  
51 250 kb (2, 3). Some indistinguishable IncI<sub>1</sub> and IncI<sub>γ</sub> plasmids have been isolated from  
52 multiple, unrelated high-risk clones, identified in different periods and bacterial species, in  
53 distant geographical areas, defined as “epidemic” plasmids (4–7). IncI pST3 plasmid is the  
54 most commonly reported *bla*<sub>CTX-M-1</sub>-IncI<sub>1</sub> plasmid in Europe, described in pathogenic human  
55 *E. coli* isolates, and in *E. coli*, and *Salmonella spp.* from food-producing animals (8). Moreover,  
56 Lucas *et al.* recently showed that resistance to Extended-Spectrum Cephalosporins (ESC) in  
57 French pigs’ *E. coli* isolates is mainly carried by highly similar *bla*<sub>CTX-M-1</sub>-IncI<sub>1</sub>/ST3 plasmids  
58 (9). IncI plasmids have been recognized in clinically relevant bacteria from human, animal, and  
59 environmental sources, and as major vehicles for the dissemination of ESBL, notably CTX-M-  
60 15 and CTX-M-1 enzymes (2, 8).

61           Previous studies using a series of experiments that included pigs inoculated with *E. coli*  
62 harboring *bla*<sub>CTX-M-1</sub>-encoding IncI plasmid showed the maintenance of the plasmid in its *E.*  
63 *coli* host, in the pig’s gut. Moreover, the loss of the plasmid was a rare event in gut microbiota  
64 during these longitudinal studies of *in vivo* carriage in pigs (10). In these models, the transfer  
65 of the *bla*<sub>CTX-M-1</sub>-encoding IncI plasmid between different *E. coli* strains occurred and  
66 underlines the dissemination capacity of the ESC resistance, independently of the exposure to

67 antibiotic drugs (11, 12). Finally, understanding the factors of IncI1 plasmids success better is  
68 important, as they are major contributors to the dissemination of ESBL genes especially in the  
69 animal reservoir.

70 The arrival of a plasmid in a new bacterium produces a highly variable fitness cost  
71 depending on the plasmid–bacterium association (13–15). Plasmid persistence and  
72 dissemination in bacterial population, under conditions that do not select for plasmid-encoded  
73 genes, are influenced by the fitness effects associated with plasmid carriage (16, 17). Previous  
74 works have shown that compensatory adaptation frequently ameliorates the fitness of costly  
75 plasmids (18–20). This compensatory evolution may play a key role in the dynamics of the  
76 spread and evolution of antibiotic resistance plasmids (13, 21–23). However, most studies on  
77 the evolutionary dynamics of plasmid–bacterium associations have been performed in  
78 laboratory-based culture systems.

79 We took the unique opportunity of the previous studies performed in pigs for probiotic  
80 testing (12), to evaluate the fitness cost of IncI pST3 plasmid on bacterial isolates collected  
81 during an *in vivo* longitudinal study of carriage of a CTX-M-1 producing *E. coli* B1 commensal  
82 strain in pigs. We also investigated the impact of IncI pST3 plasmid on the bacterial host using  
83 whole-genome sequencing (WGS) and transcriptome sequencing and analysis to determine the  
84 effect of plasmid carriage on global gene expression.

85

## 86 RESULTS

87 **Characterization of ancestral and *in vivo* evolved UB12 strains.** According to the whole-  
88 genome sequencing results, *E. coli* UB12-RC recipient strain belongs to O100:H40 serotype,  
89 B1 phylogroup, and ST2520, according to the Warwick multilocus sequence typing (MLST)  
90 scheme. This strain harbors a mutation in *rpoB* after selection in Mueller-Hinton medium (MH)  
91 containing 250 mg/liter rifampicin (10), and contains two plasmids: one P1-like phage-plasmid

92 (IncY, 98119 bp) (24), and one small mobilizable plasmid (Col156 plasmid, 5223 bp) (Table  
93 1). In addition to the IncY and Col156 resident plasmids, the UB12-TC transconjugant harbors  
94 a *bla*<sub>CTX-M-1</sub>-IncI plasmid (108699 bp), and one colicinogenic plasmid, belonging to the ColE1  
95 group (ColRNAI plasmid, 6724 bp), which disseminated from the M63-DN donor strain. The  
96 *bla*<sub>CTX-M-1</sub>-IncI plasmid is of plasmid multilocus sequence type 3 (pST3) of the IncII family  
97 and presents 99% identity and 72 % coverage to R64 plasmid, a prototype of the IncII group  
98 (ascension N° AP005147)(8). Moreover, the core genome of IncI ST3 plasmid presents with  
99 other *bla*<sub>CTX-M-1</sub>-IncII/ST3 plasmid previously described 100% identity and 99% coverage  
100 (ascension n° KJ484635)(25). The *bla*<sub>CTX-M-1</sub> gene is associated with an *ISEcp1* mobile element  
101 found at the 5' end of the gene, and the *ISEcp1*-*bla*<sub>CTX-M-1</sub> transposition unit is inserted in the  
102 shufflon region of the plasmid. Other resistance genes carried in the *bla*<sub>CTX-M-1</sub>-IncI/ST3  
103 plasmid are *dfrA17*, *sul2*, and *aadA5* (Table 1).

104 Three cefotaxime-susceptible and rifampicin-resistant isolates, collected at days 7, 18,  
105 and 20, during longitudinal carriage of the UB12-TC strain in the pigs, have spontaneously lost  
106 the IncI plasmid *in vivo* and harbor Col156, IncY, and ColRNAI plasmids (referenced as UB12-  
107 TCΔpIncI) (Table 1).

108 To identify mutations and rearrangements that occur during the carriage of UB12-TC  
109 strains in the pig's gut, we used the open-source Breseq pipeline (26) and the ancestral UB12-  
110 TC strain as reference genome. In the first step, we excluded all single nucleotide  
111 polymorphisms (SNP), insertions or deletions detected comparing mapping reads of the UB12-  
112 TC ancestral strain to the assembly of itself. In the second step, we determined genetic  
113 differences occurring between the UB12-RC recipient strain, seven UB12-TC isolates,  
114 collected in stools between day 7 and 45 after oral inoculation of pigs, i.e. *in vivo* evolved  
115 (UB12-TC<sup>ive</sup>), and the cefotaxime-susceptible and rifampicin-resistant isolates (UB12-  
116 TCΔpIncI) (Table 2). All transconjugants showed one intergenic point mutation, upstream of

117 the *nadA* gene, except two (Table 2). In chromosomal DNA, we detected seven other events  
118 between the UB12-RC recipient strain, the UB12-TC strain and six UB12-TC<sup>ive</sup>  
119 transconjugants: one intergenic point mutation between the *ygeH* and *ygeG* genes, one  
120 synonymous point mutation in the *kdsB* gene, and five nonsynonymous point mutations in the  
121 *ecpC*, *ydeE*, *ttdB*, *thiH* genes, and in the *hlyE* pseudogene (Table 2). In total, one to three point  
122 mutations per strain were detected. In plasmid DNA, a common rearrangement was detected  
123 in the shufflon region of the IncI plasmid in three transconjugants. None of other plasmids  
124 showed any modification.

125

126 **No fitness cost decrease of UB12 strains during digestive carriage in pigs.** To evaluate the  
127 IncI plasmid cost on the bacterial fitness, we compared the maximum growth rates (MGRs) of  
128 the *E. coli* M63-DN donor strain, the UB12-RC recipient strain and the ancestral UB12-TC  
129 transconjugant. The MGR of M63-DN donor strain (3.55 div h<sup>-1</sup>) was significantly higher than  
130 the MGR of UB12-RC strain (3.38 div h<sup>-1</sup>) ( $P=0.02$ ). The difference between the MGR of  
131 UB12-RC strain and the MGR of UB12-TC transconjugant (3.28 div h<sup>-1</sup>) was nonsignificant  
132 ( $P=0.09$ ) (Fig 1).

133 To evaluate the adaptation of IncI plasmid in the *E. coli* UB12 strain, we compared the  
134 MGRs of the UB12-TC strain, the M63-DN donor, and ten clones (UB12-TC<sup>ive</sup> and UB12-  
135 TC $\Delta$ pIncI) isolated from the stools of the pigs, between day 7 and day 45, during an *in vivo*  
136 longitudinal study of carriage of the UB12-TC transconjugant (11). Among these clones, three  
137 UB12-TC transconjugants had spontaneously lost the IncI plasmid (UB12-TC $\Delta$ pIncI). As no  
138 significant fitness cost variation was detected between the MGRs of the evolved UB12-TC<sup>ive</sup>  
139 transconjugants on the one hand, and between the MGRs of the UB12-TC $\Delta$ pIncI  
140 transconjugants on the other hand, further statistical analyses were performed after pooling the  
141 MGRs of the clones from each group. The difference between the MGRs of UB12-TC

142 transconjugants (3.28 div h<sup>-1</sup>) and the MGRs of UB12-TC<sup>ive</sup> evolved transconjugants (3.23 div  
143 h<sup>-1</sup>) was nonsignificant ( $P=0.08$ ), as the difference between the MGRs of UB12-RC clones and  
144 the MGRs of the UB12-TC<sup>ive</sup> transconjugants ( $P=0.07$ ). Interestingly, the differences between  
145 the MGRs of UB12-TC $\Delta$ pIncl (3.40 div h<sup>-1</sup>) and the MGRs of UB12-TC or UB12-TC<sup>ive</sup> clones  
146 were significant ( $P=0.004$  and  $P=1.61 \cdot 10^{-7}$  respectively) (Fig 1).

147

148 **No fitness cost decrease during *in vitro* experiment evolution of UB12-TC.** To assess if the  
149 absence of decrease in *in vivo* fitness cost could be reproduced *in vitro* in a constant well-  
150 defined environment, we conducted an *in vitro* evolution experiment in LB during 30 days (300  
151 generations in total) and evaluated the impact of the Incl plasmid on the bacterial host after  
152 evolution. We measured the MGRs of five independent ancestral transconjugants (UB12-TC),  
153 and of five independent clones for each lineage after *in vitro* evolution (UB12-TC<sup>ite</sup>), compared  
154 to the MGRs of five plasmid-free ancestral and evolved control strains (UB12-RC and UB12-  
155 RC<sup>ite</sup>). First, no significant fitness cost variation was detected between the MGRs of the evolved  
156 clones belonging to the same lineage. Further statistical analyses were performed after pooling  
157 the MGRs of the clones from each lineage. The differences between the MGRs of UB12-TC  
158 (3.49 div h<sup>-1</sup>) and UB12-TC<sup>ite</sup> (3.35 div h<sup>-1</sup>) clones, and the MGRs of plasmid-free control  
159 strains UB12-RC and UB12-RC<sup>ite</sup> (3.49 div h<sup>-1</sup> and 3.42 div h<sup>-1</sup>, respectively) were  
160 nonsignificant (Fig 2). Although no significant difference was found between ancestral and  
161 evolved strains, the MGRs of UB12-TC decreased after the evolution experiment (3.49 div h<sup>-1</sup>  
162 versus 3.35 div h<sup>-1</sup> for UB12-TC and UB12-TC<sup>ite</sup>, respectively). Of note, there was a trend ( $P=$   
163 0.054) for increased cost fitness in evolved strains (Fig 2).

164

165 **Description of the newly transconjugants *E. coli* isolates (NI) with Incl plasmid.** As the  
166 recipient host background could interfere in the spread of multidrug resistance plasmids, we



167 analyzed the genetic diversity of eleven cefotaxime-resistant and rifampicin-susceptible newly  
168 formed transconjugants (natural isolates, NI) harboring IncI. The phylogroups of NI are diverse  
169 (one A, B2, and D, two C, three B1, and G) (Fig 3A). Based on plasmid-families replicon, the  
170 plasmidome of NI appears varied with multiple combinations of plasmid types. Most of the  
171 eleven isolates (eight NI) carry Col-like plasmids, alone or co-carried with IncF plasmids. One  
172 isolate (NI-1) carries an IncY plasmid, sharing 98% identity and 71% coverage to the IncY  
173 plasmid of UB12-TC (Table 1). Eight NI (72%) harbor more than two plasmids, while two NI  
174 (NI-3 and NI-11) harbor the IncI plasmid alone (Table 1).

175 The core genomic divergence, estimated by the number of core genome SNPs between  
176 strains, is variable and dependent on the sequence type and phylogroup of strains, from 18513  
177 to 77175 (between *E. coli* B1 ST2520 UB12-TC strain, and respectively, *E. coli* B1 ST2540  
178 NI-4 clone and *E. coli* B2 ST95 NI-9 clone) (Fig 3A, Table S1).

179

180 **Variable cost of IncI plasmid in transconjugants *E. coli* NI from the pig's gut.** To evaluate  
181 the impact of the IncI pST3 plasmid on new bacterial host, we proposed to compare the MGRs  
182 of the UB12-TC strain, the M63-DN donor, and eleven cefotaxime-resistant and rifampicin-  
183 susceptible NI from the stools of the pigs, between day 4 and day 22. These isolates all harbored  
184 the IncI pST3plasmid that have disseminated between different *E. coli* in the pigs' microbiota  
185 after inoculation (Table 1). The MGRs of four NI were not significantly different from that of  
186 the donor strain M63-DN, while seven NI showed an MGR significantly lower than those of  
187 the M63-DN strain ( $P$  between  $2.7 \cdot 10^{-5}$  and 0.015) (Fig 3B).

188 In order to determine the relative abundance of the newly formed transconjugants in the  
189 pig's gut, we compared the titer of cefotaxime-resistant and rifampicin-susceptible *E. coli*  
190 clones to all cefotaxime-resistant *E. coli* population, on the days during which each NI was  
191 collected. We observed a variable relative abundance, ranging from 10% to 30% for NI-1, NI-

192 2, NI-3, and NI-6, from 40% to 50% for NI-4, NI-9, NI-10, and NI-11, and more than 65% for  
193 NI-5, NI-7, and NI-8.

194

195 **Transcriptomic analysis of UB12 strains.** We compared the transcriptome of the ancestral  
196 UB12-TC transconjugant with the UB12-RC recipient strain to determine the impact of plasmid  
197 acquisition on host gene expression. We also compared the transcriptome of two UB12-TC<sup>ive</sup>  
198 transconjugants, isolated between day 28 and 30 in the pig's gut, and of one UB12-TC $\Delta$ pIncI  
199 transconjugant with the UB12-RC recipient and the ancestral UB12-TC strains to determine the  
200 impact of the IncI pST3 plasmid maintenance on the bacterial host in the pig's gut. We combined  
201 the results from differential gene expression analysis of two independent biological replicates  
202 for each clone, except for UB12-TC.

203 We observed a low significant transcriptional response of the acquisition of plasmids  
204 into UB12-TC, with ten significantly ( $> 2$ -log change) differentially expressed genes (adjusted  
205 P value ( $P_{adj}$ )  $< 0.05$ ) between UB12-RC and UB12-TC strains. Six genes were down-regulated  
206 and four genes were up-regulated in UB12-TC compared to UB12-RC. Functions affected by  
207 plasmids acquisition included genes involved in mobility and chemotaxis (down-regulation of  
208 *flgB* gene; up-regulation of *motA* and *cheA* genes), in stress response (down-regulation of *cyuP*  
209 and *ydjI* genes; up-regulation of *gnsA* gene) or genes encoding for phage proteins (down-  
210 regulation of three chromosomal genes; up-regulation of one IncY plasmid-gene) (Table 3).

211 No significant change in chromosomal and plasmid gene expression was observed in the UB12-  
212 TC<sup>ive</sup> clones, compared to UB12-TC before carriage in the pig's gut.

213 However, we detected a transcriptional response between the UB12-RC and UB12-  
214 TC<sup>ive</sup> strains, with a total number of significantly ( $> 2$ -log change) differentially expressed  
215 genes ( $P_{adj} < 0.05$ ) ranging from 37 to 39. Among these differentially expressed genes observed  
216 between UB12-RC and both UB12-TC<sup>ive</sup> clones, most of down-regulated chromosomal genes

217 encoded for phage proteins (12 genes). Other down-regulated genes were *fimA* gene, encoding  
218 for type 1 fimbriae major subunit, and two genes involved in metabolism (*lpp* and *garP* genes,  
219 significantly detected down-regulated only for one of UB12-TC<sup>ive</sup> clones) (Table 3). In one or  
220 both of UB12-TC<sup>ive</sup> clones, we detected up-regulated genes involved in various stress response,  
221 such as *ariR* (oxidative stress), *bhsA* (multiple stress resistance), *yhcN* (hydrogene peroxide  
222 stress), *cspA* (temperature response), and *soxS* (superoxide stress master regulator), and genes  
223 encoding for transporters in various metabolic pathways (*srlA*, for sorbitol; *narK* or *napD*, for  
224 nitrate/nitrite; and *malEFK*, for maltose). Other functional classes of genes detected as  
225 significantly up-regulated were those involved in biofilm (*crfC* and *pdeH* genes), in mobility  
226 (*motAB* and *fliC* genes), and in chemotaxis (*cheB* and *tap* genes) (Table 3).

227 All genes involved in mobility and chemotaxis, differentially expressed between UB12-  
228 TC<sup>ive</sup> and UB12-RC strains, were also up-regulated in the UB12-TCΔpIncI clone. Moreover,  
229 other genes included in the chemotaxis system (*tar*, *cheZ<sub>YR</sub>*, and *tgr* genes) and the flagella  
230 systems (*fliS*, *flgN*, *flgKL*, and *flgM* genes) were up-regulated between UB12-TCΔpIncI and  
231 UB12-RC strains. Interestingly, *fliA*, the other major regulator of the flagella systems, appeared  
232 significantly differentially expressed (down-regulation) between UB12-TC and UB12-  
233 TCΔpIncI clones.

234 Using the replicase *repA* gene for comparison, we showed that the transcription of IncI  
235 plasmid genes was not different between UB12-TC and UB12-TC<sup>ive</sup> clones (Table S2). We  
236 observed that most of the IncI plasmid genes (111 genes, 89.5%) were expressed at low level  
237 or not expressed compared to *repA* transcription. Only 13 of predicted CDS (10%) were  
238 transcribed at level between 4- and 35-fold higher than *repA*. The most expressed plasmidic  
239 gene was annotated as a conserved protein of unknown function, located just upstream of *oriT*  
240 and the *nikA* gene (encoding for a Ribbon-helix-helix relaxosome protein), which was  
241 transcribed at level 5-fold higher than *repA*. Two other loci in plasmid core regions were

242 transcribed at level between 5- and 16-fold higher than *repA*, the *excAB* genes and an ORF  
243 located just upstream, and the *parBM* genes. Two accessory regions of the IncI plasmid  
244 encoding resistance genes were also transcribed at high level, the *ISEcp-1-bla<sub>CTX-M-1</sub>* cassette  
245 and three genes (*glmM*, *sul2*, and *drfA*) located on an *ISCR2* mobile genetic element (Table S2).  
246 We observed that the IncY plasmid genes were also expressed at low level. Only 10% (11  
247 genes) of the coding sequences were transcribed at higher level than *repA*, and all annotated as  
248 conserved proteins of unknown function (Table S2).

249  
250 **Swimming motility of strains.** To confirm the transcriptomic data on genes involved in  
251 mobility, we tested the swimming ability of all the strains used in our study in semi-solid LB  
252 agar tubes. We observed that the swimming ability decreased in UB12-TC strains compared to  
253 UB12-RC and M63-DN strains. While the ancestral UB12-TC strain presented a comparable  
254 swimming motility phenotype to all UB12-TC<sup>ive</sup> clones, UB12-TC $\Delta$ pIncI clones presented an  
255 increase in the ability to swim in semi-solid LB agar tubes. Of note, we observed various  
256 phenotypes, from non-motile to motile, for all eleven NI pig's clones, which all harbored IncI  
257 plasmid (Table 1).

258

## 259 DISCUSSION

260 Multiresistance plasmids are widely distributed across bacteria and one of the main  
261 drivers of antibiotic resistance spread. The success of a plasmid-borne resistance is constrained  
262 by the fitness cost that the multiresistance plasmid imposes on the bacterial cell. It is not clear  
263 whether epidemic *bla<sub>CTX-M-1</sub>*-IncI1 plasmids, one of the most common plasmid type in ESBL  
264 producers of animal origin (2, 8), conferred a fitness cost on the host bacterium. Previous studies  
265 have shown that the *bla<sub>CTX-M-1</sub>*-IncI1 plasmid imposes no or negligible fitness costs on its *E.*  
266 *coli* host *in vitro* (27) and persists without antimicrobial usage (28). On the other hand, using

267 *in vitro* competition experiments, Freire Martín *et al.* showed that the IncI1 plasmid-cured  
268 isolate outcompeted the original plasmid-carrying isolate, or plasmid-complemented strains,  
269 suggesting a fitness burden was imposed by carriage of this plasmid (29). These studies were  
270 performed using *in vitro* models and/or laboratory-adapted strains with limited clinical  
271 relevance. Here, we investigated the cost of a *bla*<sub>CTX-M-1</sub>-IncI1/ST3 epidemic plasmid in a  
272 commensal *E. coli* animal strain, before and after oral inoculation of pigs. In our model, *bla*<sub>CTX-</sub>  
273 *M-1*-IncI1/ST3 plasmid imposed no measurable burden on the recipient strain after conjugation  
274 and we observed no modification of the bacterial growth rate of the *bla*<sub>CTX-M-1</sub>-IncI1 plasmid-  
275 carrying strain after the longitudinal carriage in the pig's gut. However, a significant difference  
276 of bacterial growth rate was found between the *bla*<sub>CTX-M-1</sub>-IncI1/ST3 plasmid-carrying strain  
277 and the transconjugants that had spontaneously lost the IncI1 plasmid during *in vivo* carriage,  
278 suggesting a small fitness burden exists. The loss of IncI plasmid is a rare event (10), as  
279 expected because of the presence of addiction systems on these large conjugative  
280 multiresistance plasmids. As a whole, our observations could suggest that *bla*<sub>CTX-M-1</sub>-IncI1  
281 epidemic plasmids do not induce a sufficient fitness cost on its host bacterium to drive their  
282 extinction in gut microbiota, in the absence of antibiotics.

283         After ESBL-producer *E. coli* inoculation, *bla*<sub>CTX-M-1</sub>-IncI1/ST3 plasmid disseminated  
284 rapidly to natural isolates in the pig's gut, highlighting the usually very efficient conjugation  
285 system of IncI plasmids (8, 30). As reference the bacterial growth rate of the *bla*<sub>CTX-M-1</sub>-IncI  
286 donor strain, the comparative growth rate of the eleven newly formed transconjugants in the  
287 pig's gut appeared extremely variable, related to variable fitness of these IncI plasmid-carrying  
288 new spontaneous transconjugants. Although it is not possible to determine the competitiveness  
289 of the host cells before IncI plasmid acquisition due to the experimental design of our study,  
290 this suggests that the fitness cost could not act as a strong barrier to transmission of the *bla*<sub>CTX-</sub>  
291 *M-1*-IncI1 epidemic plasmid, among natural populations of *E. coli* in a relevant environmental

292 niche such as the gut. Interestingly, the relative abundance of some of the newly formed  
293 transconjugants was greater than 50%, suggesting that these clones represented dominant ESC-  
294 resistant *E. coli* strains in the pig's gut at this time.

295 Previous studies have reported *in vivo* transfers of multidrug-resistant plasmids despite  
296 competition from fecal flora, and a high risk of rapid increase of transconjugants becoming the  
297 dominant population under antibiotic treatment (30–32). Some studies have shown that  
298 antibiotics exposure increased conjugative transfer of *bla*<sub>CTX-M-1</sub>-IncI plasmids (33) and it has  
299 been suggested that the transfer rate of the IncI plasmid may be underestimated in *in vitro* study  
300 models (34). The high predominance and worldwide spread of some IncII plasmids, especially  
301 among animal ESBL-producer *E. coli*, could be explained by a low fitness cost imposed on the  
302 new recipient strain, combined with a high conjugation efficiency and potentiated by a high  
303 exposure of animals to antibiotics in agricultural settings.

304 A complex plasmid-chromosome cross-talk is involved in plasmid domestication into  
305 their host cells and often appears to be dependent of the plasmid-host pairing studied. Among  
306 these plasmid-host adaptation effects associated with a potential fitness benefit, only changes  
307 in chromosomal gene expression have been observed in some plasmid-host evolutions (35).  
308 These modifications in the expression of chromosomal genes for the bacterium adaptation to  
309 the acquisition of the plasmid appeared highly variable both in the degree of alteration and in  
310 the range of cellular functions that are affected (35–40). However, chromosome-encoded  
311 functions most commonly targeted by plasmid carriage include respiration, signaling, energy  
312 production, various metabolism pathways, and virulence factors, especially adhesion-related  
313 functions involved in biofilm formation and mobility (41, 42). To analyse the impact of the  
314 *bla*<sub>CTX-M-1</sub>-IncII/ST3 plasmid on the commensal *E. coli* host, we performed WGS and RNA-  
315 sequencing of the ancestral strains on the one hand, and UB12 isolates collected from pigs'  
316 feces on the other hand. In our study, a very small number of SNPs in chromosomal and plasmid

317 coding sequences were detected in the UB12 isolates, without clear convergence. In addition,  
318 RNA-sequencing revealed that only the expression of a small set of chromosomal genes was  
319 altered in the host cell after acquisition of the IncI ST3 plasmid, involving chromosomal genes  
320 encoding prophages proteins and genes found in different processes such as metabolism, stress  
321 response, and cell mobility. We showed that the transcriptional expression of chemotaxis and  
322 flagellar genes change between IncI plasmid-carrying and plasmid-free UB12 isolates collected  
323 after oral inoculation of pigs, in accordance with the modification in the swimming capacities  
324 of these clones. None of mutations observed in our data were associated with change in gene  
325 expression, suggesting that we observed a physiological response. It is interesting to note that  
326 chromosomal genes implicated in cell mobility are also involved in the bacterial response to  
327 environmental changes especially in the highly dynamic and compartmentalized gut  
328 environment (43). In our model, plasmid-host adaptation could be the result of both the specific  
329 plasmid-host pairing studied and adaptation to environmental changes in the pig's gut. Overall,  
330 we observed that the *bla*<sub>CTX-M-1</sub>-IncI1/ST3 plasmid has only a limited effect on the host cell,  
331 targeting cellular functions classically known to be affected in the host bacterium after plasmid  
332 acquisition.

333 We observed that most of the *bla*<sub>CTX-M-1</sub>-IncI1/ST3 plasmid genes were expressed at low  
334 level or not expressed in host cell. Our observation indicates that the IncI plasmid transcription  
335 is highly regulated in this model, which is consistent with findings from other studies reporting  
336 transcriptional profiles of other large conjugative plasmids (39). Recent studies have reported  
337 the involvement of plasmid genes encoding for nucleoid-associated proteins (NAPs) homologs,  
338 such as H-NS-like proteins, in transcriptional regulation networks between plasmids and host  
339 chromosome (42, 44–46). Plasmids encoding H-NS-like “stealth” protein reduce their fitness  
340 cost by silencing transcriptional activities of their own genes, as well as some chromosomal  
341 genes, and therefore minimize the impact of plasmid introduction in the bacterium. Large



342 conjugative plasmids frequently carry NAP gene homologs but, using *in silico* analysis, similar  
343 genes were not yet identified in plasmids of the IncI incompatibility group (47). However, a  
344 large number of IncI backbone genes are yet annotated as hypothetical proteins. Future studies  
345 could be interesting to characterize new regulators still unknown among these genes, and to  
346 better understand the cross-talk between IncI plasmids and the host chromosome.

347 Our study has further limitations. First, we performed the experiments in a unique  
348 commensal *E. coli* host strain and the genetic background of strains plays an important role in  
349 plasmid-host adaptation. Second, due to the design of our study, we used *in vitro* plating of the  
350 bacteria and therefore we could not directly observe the *E. coli* populations of interest in the  
351 pig's gut. This approach might have introduced a bias as a consequence of short adaptation of  
352 the IncI plasmid in the new recipient strain. Nevertheless, our study is the first to provide direct  
353 evidence of *in vivo* plasmid transfer in the pig's gut and to confirm that *bla*<sub>CTX-M-1</sub>-IncI1/ST3  
354 plasmid fitness cost on its bacterial hosts is negligible *in vivo*, in a relevant environmental niche  
355 such as the gut.

356

357

## 358 MATERIALS AND METHODS

359 ***Bacterial strains and plasmids.*** For this study, a total of 24 strains were selected from a pig  
360 strain collection of ANSES laboratory (Ploufragan, France). The *E. coli* transconjugant UB12-  
361 TC was obtained by conjugation between a randomly chosen pan-susceptible pig commensal  
362 *E. coli* isolate, made resistant to 250 mg/liter rifampicin (recipient strain UB12-RC, B1,  
363 O100:H40) and an ESBL-resistant *E. coli* pig isolate (donor strain M63-DN, B1, O147:H7).  
364 Each strain belongs to ST2520 and ST2521, respectively, according to the Warwick MLST  
365 scheme.

366 Thus, *E. coli* UB12-TC is resistant to rifampicin and harbors a IncI1 pST3 plasmid encoding  
367 *bla*<sub>CTX-M-1</sub>, *sul2*, *dfrA17*, and *aadA5* genes. Seven cefotaxime- and rifampicin-resistant isolates  
368 (named UB12-TC<sup>ive</sup>), three cefotaxime-susceptible and rifampicin-resistant isolates (named  
369 UB12-TCΔpIncI), and eleven cefotaxime-resistant and rifampicin-susceptible isolates (named  
370 NI-1 to NI-11) were chosen among all strains collected during a series of experiments conducted



371 to evaluate the carriage of ESBL-producing *E. coli* in the pig's gut (11, 12). These isolates have  
372 been collected from day 4 to day 45 after pig inoculation.

373

374 ***Animals and experimental design.*** As previously described in Mourand *et al.* (10, 12), the  
375 experiments were conducted in the ANSES, Ploufragan, France, animal facilities. The trials  
376 were conducted with 6- to 8-week-old specific pathogen-free and initially ESC-resistant *E. coli*-  
377 free Large White piglets. The animals were randomized before the experiment. They did not  
378 receive any antibiotic treatment prior to the trial and were given the same non-supplemented  
379 feed. Each room contained eight pigs placed in two pens of four animals each. For both trials,  
380 UB12-TC was inoculated on the first day (day 0): each pig was orally given a 10-ml suspension  
381 prepared from strain cultivated on MH agar containing cefotaxime (2 mg/liter). Individual fecal  
382 samples were collected from animals on day 0 before inoculation, and between day 1 and 45.  
383 The fecal samples were stored at -70°C until analysis.

384 The numbers of cefotaxime-resistant *E. coli* (including UB12-TC and NI) in the individual fecal  
385 samples were estimated by spreading 100 µl of 10-fold dilutions on MacConkey agar plates  
386 (containing respectively 2 mg/liter cefotaxime), in triplicate. The titers of total *E. coli*  
387 population were determined by plating serial dilutions on MacConkey agar plates without  
388 antibiotic. After incubation at 37°C, the colonies obtained on supplemented or non-  
389 supplemented MacConkey agar plates were enumerated, and the titers were calculated for each  
390 pig per day. The relative abundance of cefotaxime- and rifampicin-susceptible (NI clones) was  
391 calculated based on the ability of 2 to 5 isolates obtained from each pig on each day on  
392 cefotaxime-supplemented media to grow on rifampicin-supplemented media, and expressed as  
393 a percentage.

394

395 ***Antimicrobial susceptibility testing.*** Antimicrobial susceptibility testing by the disk diffusion  
396 method (ampicillin, ticarcillin, piperacillin, amoxicillin-clavulanic acid, ticarcillin-clavulanic  
397 acid, piperacillin-tazobactam, cefotaxime, ceftazidime, aztreonam, cefoxitin, cefepime,  
398 ertapenem and imipenem, association of sulphonamides + trimethoprim, tetracycline,  
399 streptomycin, nalidixic acid, ciprofloxacin, and rifampicin) was performed according to  
400 European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines  
401 (<http://www.eucast.org>).

402

403 ***Motility assay.*** Donor strain M63-DN, recipient strain UB12-RC, ancestral UB12-TC, all  
404 UB12-TC<sup>ive</sup> and UB12-TCΔpIncI clones were grown overnight in LB medium, washed,

405 suspended in isotonic water to a concentration of  $1 \times 10^2$  cells ml<sup>-1</sup> and used to inoculate semi-  
406 solid LB agar tubes containing 0.35% agar, incubated for 48 h at 37°C. Experiments were  
407 repeated 3 times. For data analysis, three categories were considered (motile, partially motile,  
408 and nonmotile).

409

410 ***In vitro evolution experiments.*** Five randomly selected colonies of UB12-RC and UB12-TC  
411 were inoculated into 5 ml of LB and grown under constant shaking at 200 rpm. After overnight  
412 incubation at 37°C, 5 µl of each culture was inoculated into five tubes containing 5 ml of fresh  
413 LB. At this step, the 10 cultures were considered the ancestors of each lineage (day 0 of the  
414 evolution assay). Next, the 10 cultures were propagated by daily serial transfer, with a 1/1,000  
415 dilution (5 µl of culture grown overnight diluted into 5 ml of fresh LB without antibiotic), for  
416 30 days (corresponding to 300 generations in total) and incubated overnight at 37°C under  
417 constant stirring at 200 rpm. Finally, after 30 days, 10 independent lineages were obtained  
418 (denoted “ite”). At day 0 and every 5 or 6 days of the evolution assay, the culture was isolated  
419 on LB agar for purity, and antibiotic susceptibility was tested by disk diffusion. A culture  
420 sample from each lineage was collected and stored at -80°C with glycerol for future analysis.

421

422 ***Individual fitness assays.*** Fitness, defined here as the Maximum Growth Rate (MGR), was  
423 determined from growth curves using a high-precision technique developed in-house as  
424 described previously (18). For each strain, the fitness assay was repeated six times. The  
425 measurements were collected using a Python script developed in our laboratory, and the MGR  
426 was obtained by the `lm` function in R (<http://www.R-project.org/>).

427

428 ***Whole-genome sequencing.*** WGS was performed using Illumina technology. Briefly, DNA  
429 was prepared for paired-end sequencing on the Illumina MiniSeq (Integragen, MA, USA) for  
430 all strains. DNA samples were extracted using the genomic DNA NucleoMag tissue kit from  
431 Macherey-Nagel (Hoerd, France). DNA libraries were prepared and indexed, and tagmentation  
432 was processed using the Nextera DNA Flex library Prep kit (Illumina, USA). The sample  
433 concentrations were normalized in equimolar concentration at 1 nM. The pooled libraries were  
434 sequenced to a read length of 2 by 150 bp with Illumina MiniSeq High output reagent Kit. The  
435 genomes were sequenced at an average depth of 50X.

436

437 **DNA sequence analysis and UB12-TC genome comparison.** Illumina genome sequence reads  
438 were assessed for quality using FastQC v0.11.8 and subsequently trimmed with a cutoff phred  
439 score of 30 using TrimGalore v0.4.5. All genomes were *de novo* assembled using SPAdes  
440 v.3.13.1 software and analysed with an in-house bioinformatics pipeline  
441 (<https://github.com/iame-researchCenter/Petanc>) adapted from Bourrel et al. (48). Briefly,  
442 genome typing was performed including MLST determination, phylogrouping, and serotyping.  
443 The pipeline was also used to get information about plasmid replicons, resistance and virulence  
444 genes, the prediction of a plasmid sequence or a chromosome sequence for each contig using  
445 PlaScope (49).

446 For each sample, sequenced reads were mapped to a reference sequence to analyse the  
447 mutations and rearrangements. For reference plasmid sequences, we used the plasmid  
448 sequences extracted from GenBank (<http://www.ncbi.nlm.nih.gov/GenBank/>) (ColRNAI,  
449 accession n° J01566; IncY, accession n° MH42254; Col156, accession n° EU090225 and IncI,  
450 accession n° KJ484635) and *de novo* assembled contigs as predicted to plasmid genome of  
451 UB12-TC strain with PlaScope, included in our pipeline. For reference chromosomal genome,  
452 first, we calculated the mash distance between UB12-TC genome sequence and *E. coli* genomes  
453 from RefSeq database using Mash v.2.0 and we selected the closest *E. coli* strain assembly  
454 ASM190094v1. Then, all predicted chromosomal contigs of the UB12-TC genome were  
455 rearranged as scaffold with RAGOUT v2.3 (<https://github.com/fenderglass/Ragout>)(50) and  
456 the reference genome (*E. coli* assembly ASM190094v1). All scaffolds of the UB12-TC strain  
457 obtained were annotated on the MicroScope platform (51). Identification of the point mutations  
458 and rearrangements between UB12-TC and UB12-TC<sup>ive</sup> were performed using the open  
459 computational pipeline Breseq v0.27.1 (26). All SNPs identified in the ancestor clones that were  
460 sequenced were subtracted from the sequences of the evolved clones.

461

462 **Transcriptome sequencing.** RNA sequencing was performed on the recipient strain UB12-RC,  
463 the ancestral transconjugant UB12-TC, two evolved UB12-TC transconjugants (UB12-TC<sup>ive</sup>,  
464 detected in pig' stools at day 28 and 31) and one UB12-TC transconjugant that had lost the  
465 plasmid IncI (UB12-TC $\Delta$ IncI, detected in pig' stools at day 20).

466 For RNA extraction, a total of 10 cultures representing two biological replicates per strain were  
467 independently grown. For each strain, 6  $\mu$ l of an overnight culture was added in to 6 ml of fresh  
468 LB and was incubated at 37°C under constant stirring at 200 rpm until reaching an OD600 of  
469 approximately 0.6. 3 ml of each culture were taken, representing two technical replicates, and

470 mixed with RNA protect (Qiagen, Courtaboeuf, France) according to the manufacturer's  
471 recommendations. Cells were immediately pelleted by a 10 min centrifugation at 5,000 g. RNA  
472 was prepared using the Quick-RNA Fungal/Bacterial MiniPrep kit (Ozyme, ZymoResearch,  
473 USA). DNA was digested using Turbo DNase (Thermofisher, UK) based on the  
474 manufacturer's protocol. Verification of complete removal of any contaminating DNA was  
475 performed by PCR amplification of *recA* housekeeping gene. The final RNA solution was  
476 quantified with the Qubit<sup>TM</sup> RNA High Sensitivity assay kit (Thermofisher Scientific, UK) and  
477 RNA quality was evaluated using the Agilent 2100 Bioanalyzer RNA 6000Nano Kit (Agilent  
478 Technologies, Santa Clara, CA). The RNA was immediately stored at -80°C.

479 For each replicate, 8 µl of RNA was treated to remove 16S and 23S rRNAs and cDNA libraries  
480 were prepared using the Zymo-Seq RiboFree<sup>®</sup> Total RNA Library Kit (Ozyme,  
481 ZymoResearch, USA). The cDNA quantity was determined with the Qubit<sup>TM</sup> dsDNA Broad  
482 Range assay kit. Paired-end sequencing of the pooled libraries was performed with an Illumina  
483 MiniSeq Mid Output reagent kit and an Illumina NextSeq 500/550 High Output Kit v2.5  
484 (Illumina, USA), configured to 2 X 150-bp cycles, yielding approximately 8 and 400 million  
485 reads, respectively.

486

487 **Transcriptome analysis.** To assure high sequence quality, the Illumina cDNA reads in FASTQ  
488 format were trimmed to remove all sequencing adapters using TrimGalore v0.4.5., according  
489 to the manufacturer's recommendations. Then, cDNA reads were trimmed again, using  
490 TrimGalore v.0.4.5., with a cutoff phred of 30, and the reads shorter than 70 bp were discarded.  
491 A final quality check was done using FastQC v0.11.8. Reads were then mapped to the UB12-  
492 TC reference genome sequence (see above) using BWA alignment software and BWA-MEM  
493 algorithm v.0.7.17. (52) Mapping statistics were verified using BAMStat v.1.25. and SAMtools  
494 idxstat v.1.9. (53). Reads counts were performed using featureCounts v.2.0.3.  
495 (<http://subread.sourceforge.net>) (54). An R package, DESeq2 v.1.34. was used to normalize  
496 and to identify differentially expressed genes between each sample  
497 (<http://www.bioconductor.org/packages/release/bioc/html/DESeq2.html>) (55). A cutoff of q  
498 value of < 0.05 and a fold change of > 2 were used to measure statistical significance.

499

500 **Statistics.** For statistical data analysis from the fitness assays, we used an analysis of variance  
501 (ANOVA) and unpaired Student's test using R software, with *P* values of < 0.05 being  
502 considered significant.

503

504 **Ethics.** The experiments were performed in accordance with French animal welfare regulations,  
505 and the protocol was approved by the ANSES/ENVA/UPEC ethical committee (ComEth  
506 authorizations 12-032 and 15-050 [no. APAFIS: 2015061813246553]). The experiments were  
507 conducted in the ANSES, Ploufragan, France, animal facilities.

508  
509 **Data availability.** All reads have been deposited at the European Nucleotide Archive (project  
510 accession number PRJEB8070, ID samples from ERS14303872 to ERS14303895)

511  
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715 **Fig 1** : Boxplot representation of the maximum growth rate (MGR) of each pool lineage: MGR  
716 of the donor strain (M63-DN), the recipient strain (UB12-RC), the ancestral transconjugant  
717 (UB12-TC, IncI, pST3), seven *in vivo* evolved transconjugants (UB12-TC<sup>ive</sup>), and three evolved  
718 transconjugants which had lost *in vivo* the IncI plasmid (UB12-TCΔpIncI). UB12-TC<sup>ive</sup> and  
719 UB12-TCΔpIncI were isolated in stools between day 7 and day 45 during longitudinal study of  
720 *in vivo* carriage in pigs. For each box, the central mark indicates the median, and the bottom  
721 and top edges of the box indicate the 25th and 75th percentiles, respectively. Each point  
722 corresponds to one measurement per clone, which were repeated six times per strains. Asterisks  
723 indicate significant differences (\* =  $P < 0.05$ , \*\* =  $P < 0.01$  and \*\*\* =  $P < 0.001$ ). NS = not  
724 significant.

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726 **Fig 2** : Boxplot representation of the maximum growth rate (MGR) of each pool lineage: MGR  
727 of five ancestral UB12-RC and UB12-TC control strains and evolved UB12-RC and UB12-TC  
728 strains at day 30. For each box, the central mark indicates the median, and the bottom and top  
729 edges of the box indicate the 25th and 75th percentiles, respectively. Each point corresponds to  
730 one measurement per clone, which were repeated six times per transconjugant and control. NS  
731 = not significant.

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733 **Fig 3** : (A), Phylogenetic tree of the 11 newly formed transconjugants detected among pig *E.*  
734 *coli* commensal isolates (NI clones, *E. coli*, IncI, pST3), the M63-DN donor strain, and the  
735 UB12-TC transconjugant strain (in bold) are presented. The tree is rooted on an *Escherichia*  
736 clade (E1492cladeI, (56)). The phylogenetic tree was constructed with IQ-TREE v1.6.9 from  
737 the core genome genes using Roary v1.007002. From left to right are presented the Sequence  
738 Type (according to the Warwick University scheme), the O and H serotypes, the *fimH* allele,  
739 and the fitness cost of NI clones compared to the donor strain (M63-DN) (NA= not applicable,  
740 NS= not significant, \* =  $P < 0.05$ , \*\* =  $P < 0.01$  and \*\*\* =  $P < 0.001$ ). Each branch of the same  
741 phylogenetic group are colored (yellow, D; red, B2; orange, G; blue, A; light green, C; dark  
742 green, B1). Information on O group, H type, and *fimH* allele are given in Table 1. (B), Boxplot  
743 representation of the maximum growth rate (MGR) of each strain : donor strain (M63-DN),  
744 UB12-TC ancestral transconjugant and NI clones, isolated in stools between day 4 and day 22  
745 during longitudinal study of *in vivo* carriage in pigs. For each box, the central mark indicates  
746 the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles,  
747 respectively. Each point corresponds to one measurement per clone, which were repeated six  
748 times per strains. Asterisks in red indicate significant differences (\* =  $P < 0.05$ , \*\* =  $P < 0.01$

749 and \*\*\* =  $P < 0.001$ ) in comparison to M63-DN. Colors correspond to the strains of the same  
750 phylogroup (color code as in A)

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**Table 1:** Characterization of donor *E. coli* strain, evolved and ancestral *E. coli* UB12 clones, and new in vivo transconjugants used in this study.

status	Isolate name	Experiment (month/year)	Box <sup>a</sup>	Animal <sup>a</sup>	Day of evolution	Phylogroup	MLST type <sup>b</sup>	Serotype	FimH Allele	Plasmid replicon <sup>c</sup>	Antimicrobial resistance genes	Mobility <sup>d</sup>
Ancestral strains	M63-DN		-	-	-	B1	ST2521	O147:H7	fimH31	<b>Incl (KJ484635)</b> ; ColRNAI (J01566)	<i>bla</i> <sub>CTX-M-1</sub> , <i>sul2</i> , <i>aadA5</i> , <i>dfra17</i>	+
	UB12-RC		-	-	-	B1	ST2520	O100:H40	fimH31	<b>IncY (MH42254)</b> ; Col156 (EU090225)		+
	UB12-TC		-	-	J0	B1	ST2520	O100:H40	fimH31	<b>Incl (KJ484635)</b> ; ColRNAI (J01566); <b>IncY (MH42254)</b> ; Col156 (EU090225)		+/-
Evolved strains with Incl plasmid	UB12-TC <sup>LIVE</sup> .1	January/2013	F3v	4368	D28	B1	ST2520	O100:H40	fimH31	<b>Incl (KJ484635)</b> ; ColRNAI (J01566); <b>IncY (MH42254)</b> ; Col156 (EU090225)	<i>bla</i> <sub>CTX-M-1</sub> , <i>sul2</i> , <i>aadA5</i> , <i>dfra17</i>	+/-
	UB12-TC <sup>LIVE</sup> .2	June/2013	B2d	238	D8							+/-
	UB12-TC <sup>LIVE</sup> .3	June/2013	B4v	4631	D31							+/-
	UB12-TC <sup>LIVE</sup> .4	October/2012	B1	4219	D7							+/-
	UB12-TC <sup>LIVE</sup> .5	October/2012	B1	4219	D20							+/-
	UB12-TC <sup>LIVE</sup> .6	December/2015	C3d	462	D18							+/-
	UB12-TC <sup>LIVE</sup> .7	December/2015	C4v	5652	D45							+/-
Evolved strains with Incl plasmid loss	UB12-TCApIncl.1	June/2013	B2v	4613	D20	B1	ST2520	O100:H40	fimH31	ColRNAI (J01566); <b>IncY (MH42254)</b> ; Col156 (EU090225)	-	+
	UB12-TCApIncl.2	April/2012	C3v	3716	D7							+
	UB12-TCApIncl.3	April/2012	C3v	3716	D18							+
New in vivo transconjugants with Incl plasmid	NI.1	January/2013	B2v	4331	D22	D	ST13022	-:H25	fimH123	<b>Incl (KJ484635)</b> ; <b>IncY (MH42254)</b> ; <b>IncFII (pRSB107)</b>	<i>bla</i> <sub>CTX-M-1</sub> , <i>sul2</i> , <i>aadA5</i> , <i>dfra17</i>	+
	NI.2	January/2013	F3v	4324	D22	A	ST48	O184:H11	FimH41	<b>Incl (KJ484635)</b> ; <b>IncFIC (FII)</b> , <b>IncFIB (AP001918)</b> ; <b>IncFII (pSFO) (AF401292)</b> ; ColE10 (X01654); ColRNAI (J01566)	<i>bla</i> <sub>CTX-M-1</sub> , <i>sul2</i> , <i>aadA5</i> , <i>dfra17</i> , <i>sul1</i> , <i>tetA</i> , <i>tetB</i>	+/-
	NI.3	January/2013	F3v	4368	D10	B1	ST13023	-:H7	fimH68	<b>Incl (KJ484635)</b>	<i>bla</i> <sub>CTX-M-1</sub> , <i>sul2</i> , <i>aadA5</i> , <i>dfra17</i>	+
	NI.4	June/2013	B2v	4591	D16	B1	ST2540	OgN9:H2	fimH31	<b>Incl (KJ484635)</b> ; ColRNAI (J01566)	<i>bla</i> <sub>CTX-M-1</sub> , <i>sul2</i> , <i>aadA5</i> , <i>dfra17</i>	+
	NI.5	June/2013	B2v	4575	D13	G	ST657	O149:H25	fimH97	<b>Incl (KJ484635)</b> ; <b>IncFII (pRSB107) (AJ851089)</b> ; ColRNAI (J01566); Col(MG828)	<i>bla</i> <sub>CTX-M-1</sub> , <i>sul2</i> , <i>aadA5</i> , <i>dfra17</i>	+
	NI.6	June/2013	B2d	238	D8	C	ST410	Onovel14:H9	fimH24	<b>Incl (KJ484635)</b> ; <b>IncFIC (FII)</b> , <b>IncFIB (AP001918)</b> , <b>IncFII (pSFO) (AF401292)</b> ; ColRNAI (J01566)	<i>bla</i> <sub>CTX-M-1</sub> , <i>sul2</i> , <i>aadA5</i> , <i>dfra17</i> , <i>tetA</i>	-
	NI.7	October/2012	B1	4219	D20	G	ST117	O24:H4	fimH97	<b>Incl (KJ484635)</b> ; <b>IncFIC (FII)</b> , <b>IncFIB (AP001918)</b> , <b>IncFII (pSFO) (AF401292)</b> ; ColRNAI (J01566); Col(MG828)	<i>bla</i> <sub>CTX-M-1</sub> , <i>sul2</i> , <i>aadA5</i> , <i>dfra17</i>	+
	NI.8	December/2015	C4d	5625	D4	B1	ST767	O8:H9	fimH32	<b>Incl (KJ484635)</b> ; <b>IncFIC (FII)</b> , <b>IncFIB (AP001918)</b> ; <b>IncFII (pSFO) (AF401292)</b> ; Col(MG828)	<i>bla</i> <sub>CTX-M-1</sub> , <i>sul2</i> , <i>aadA5</i> , <i>dfra17</i> , <i>tetA</i>	+
	NI.9	December/2015	C4d	5625	D11	B2	ST95	O18:H7	fimH15	<b>Incl (KJ484635)</b> ; <b>IncFIC (FII)</b> , <b>IncFIB (AP001918)</b> , <b>IncFII (pSFO) (AF401292)</b> ; ColRNAI (J01566)	<i>bla</i> <sub>CTX-M-1</sub> , <i>sul2</i> , <i>aadA5</i> , <i>dfra17</i>	+/-
	NI.10	December/2015	C4v	5626	D11	G	ST117	-:H19	fimH97	<b>Incl (KJ484635)</b> ; <b>IncFIB (AP001918)</b> , <b>IncFII (pRSB107)</b> ; Col(MG828); ColB282 (DQ995353); p0111; ColRNAI (J01566)	<i>bla</i> <sub>CTX-M-1</sub> , <i>sul2</i> , <i>aadA5</i> , <i>dfra17</i> , <i>tetB</i>	-
	NI.11	December/2015	C4d	5651	D11	C	ST88	O8:H9	fimH39	<b>Incl (KJ484635)</b>	<i>bla</i> <sub>CTX-M-1</sub> , <i>sul2</i> , <i>aadA5</i> , <i>dfra17</i>	-/+

<sup>a</sup>, detailed in (9, 11); <sup>b</sup>, according to the Warwick multilocus sequence typing (MLST) scheme; <sup>c</sup>, PlasmidFinder, **Incl** plasmid is in bold; <sup>d</sup>, (+) total spreading within medium, (+/-) partial spreading, and (-) not spreading

**Table 2** : Characterization of mutations and rearrangements in the chromosome and plasmids of ancestral and evolved *E. coli* UB12 clones.

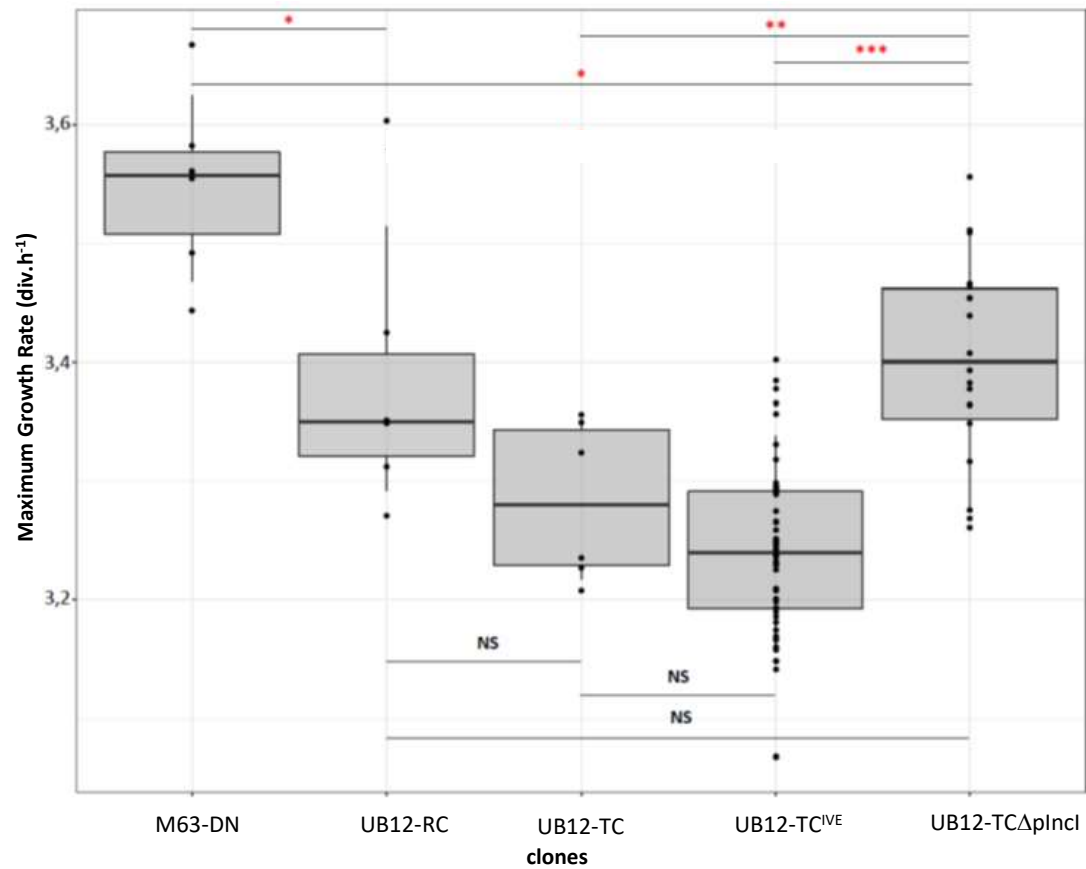
Strains	Mutations <sup>a</sup>		Rearrangements <sup>b</sup>	
	Plasmids	Chromosome	Plasmids	Chromosome
UB12-RC (recipient)	ΔpIncl, ΔCol156	-	-	-
UB12-TC (ancestral tranconjugant)	-	intergenic region (-286/-); quinolinate synthase gene ( <i>nadA</i> ) / (T→A)	-	-
UB12-TC <sup>IVE</sup> .1	-	intergenic region (-286/-); quinolinate synthase gene ( <i>nadA</i> ) / (T→A) putative fimbrial usher protein EcpC ( <i>ecpC</i> ) / V340L ( <u>G</u> TG→ITG)	pIncl : -/Mobile element protein, ISEcp1/ Tryptophan synthase beta chain like/Incl1 plasmid conjugative transfer pilus-tip adhesin protein PilV	-
UB12-TC <sup>IVE</sup> .2	-	intergenic region (-286/-); quinolinate synthase gene ( <i>nadA</i> ) / (T→A)	-	-
UB12-TC <sup>IVE</sup> .3	-	intergenic region (-286/-); quinolinate synthase gene ( <i>nadA</i> ) / (T→A)	-	-
UB12-TC <sup>IVE</sup> .4	-	-	pIncl : Tryptophan synthase beta chain like/Incl1 plasmid conjugative transfer pilus-tip adhesin protein PilV	-
UB12-TC <sup>IVE</sup> .5	-	intergenic region (-286/-); quinolinate synthase gene ( <i>nadA</i> ) / (T→A) intergenic region (-207/+128); putative transcriptional regulator ( <i>ygeH</i> ) / TPR repeat-containing putative chaperone ( <i>ygeG</i> ) / (T→C)	pIncl : -/Mobile element protein, ISEcp1/ Tryptophan synthase beta chain like/Incl1 plasmid conjugative transfer pilus-tip adhesin protein PilV	-
UB12-TC <sup>IVE</sup> .6	-	intergenic region (-286/-); quinolinate synthase gene ( <i>nadA</i> ) / (T→A) dipeptide exporter ( <i>ydeE</i> ) / R23H (C <u>G</u> C→C <u>A</u> C) 3-deoxy-manno-octulosonate cytidyltransferase ( <i>kdsB</i> ) / <b>R38R</b> (C <u>G</u> C→C <u>G</u> A)	-	-
UB12-TC <sup>IVE</sup> .7	-	intergenic region (-286/-); quinolinate synthase gene ( <i>nadA</i> ) / (T→A) L(+)-tartrate dehydratase subunit beta ( <i>ttdB</i> ) / E110K ( <u>G</u> AA→ <u>Δ</u> AA)	-	-
UB12-TCΔpIncl.1	ΔpIncl	intergenic region (-286/-); quinolinate synthase gene ( <i>nadA</i> ) / (T→A)	-	-
UB12-TCΔpIncl.2	ΔpIncl	2-iminoacetate synthase ( <i>thiH</i> ) / A147S ( <u>G</u> CC→ <u>I</u> CC)	-	-
UB12-TCΔpIncl.3	ΔpIncl	intergenic region (-286/-); quinolinate synthase gene ( <i>nadA</i> ) / (T→A) fragment of hemolysin E (pseudogene, <i>hlyE</i> ) / (C→A)	-	-

<sup>a,b</sup> Mutations and rearrangements are detected in the chromosome or plasmids of each strain compared to the UB12-RC genome ; Nucleotide changes are given in parentheses and underlined; synonymous mutations are in bold; the position of intergenic mutations is shown by the relative distance with start or stop codon of genes; Δ = deletion

**Table 3** : List of differentially expressed genes from UB12-TC clones compared to UB12-RC recipient strain.

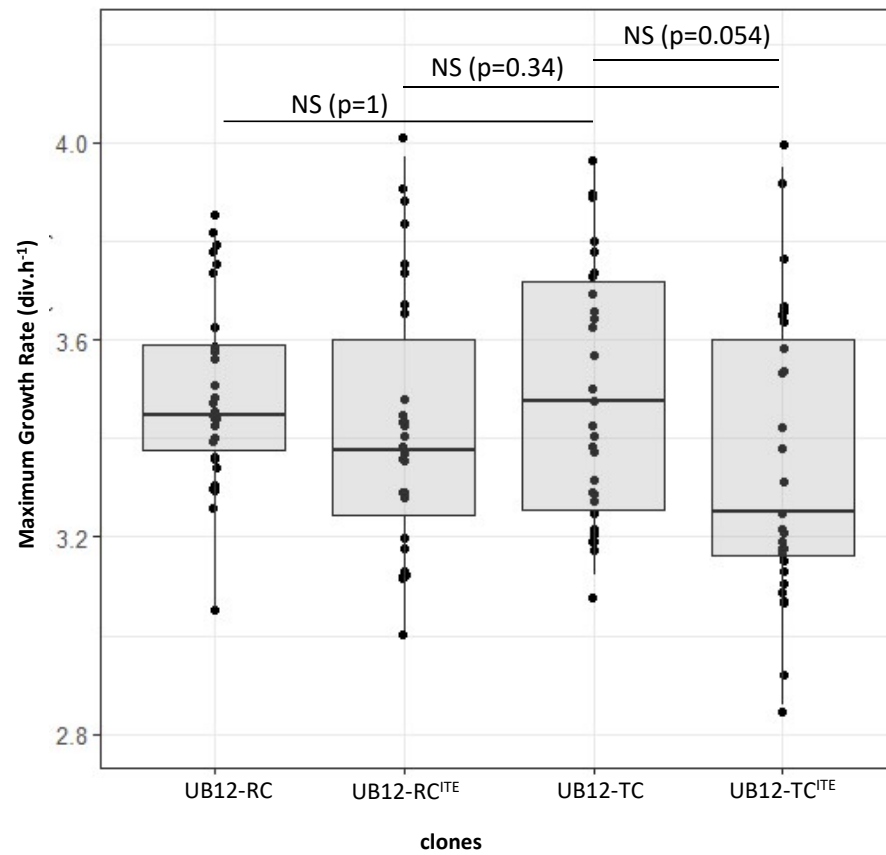
Genome Reference	Locus Reference	Gene	Product	GO Terms <sup>a</sup>	UB12-RC		UB12-TC		UB12-TC <sup>ve</sup> .1		UB12-TC <sup>ve</sup> .3		UB12-TC <sup>ve</sup> incl.1	
					log2FC	P <sub>adj</sub>	log2FC	P <sub>adj</sub>	log2FC	P <sub>adj</sub>	log2FC	P <sub>adj</sub>	log2FC	P <sub>adj</sub>
ECOL4764K	85852650	<i>cheA</i>	chemotaxis protein CheA	Chemotaxis (NT)	2.44783641	0.00237824	1.6618073	0.02643089	1.61793383	0.05792439	3.39694919	3.1361E-17		
ECOL4764K	85852652	<i>tar</i>	methyl-accepting chemotaxis protein Tar	Chemotaxis (NT)	0.35904909	0.99975668	0.90038027	0.56711099	1.08193808	0.12425088	2.52614687	1.8061E-09		
ECOL4764K	85852654	<i>cheR</i>	chemotaxis protein methyltransferase	Chemotaxis (J)	1.31467434	0.99975668	0.53731584	0.99726782	0.74834092	0.94241864	1.94461227	2.5904E-05		
ECOL4764K	85852655	<i>cheB</i>	protein-glutamate methyl-esterase/protein glutamine deamidase	Chemotaxis (NT)	1.61017833	0.86086269	1.95395442	0.00038597	1.90873908	0.02550016	3.37827951	4.9075E-13		
ECOL4764K	85852656	<i>cheY</i>	chemotaxis protein CheY	Chemotaxis (KT)	-0.38604911	0.99975668	0.84638707	0.72815763	0.84992797	0.41766744	1.44825921	0.00533935		
ECOL4764K	85852657	<i>cheZ</i>	chemotaxis protein CheZ	Chemotaxis (J)	0.14320588	0.99975668	0.47044846	0.99726782	0.53222483	0.99982751	1.46782071	0.01350632		
ECOL4764K	85853147	<i>trg</i>	methyl-accepting chemotaxis protein Trg	Chemotaxis (NT)	0.22621629	0.99975668	0.47635971	0.99726782	0.34401042	0.99982751	1.32683919	0.01396423		
ECOL4764K	85852653	<i>tap</i>	methyl-accepting chemotaxis protein Tap	Chemotaxis (NT)	1.64986384	0.27943748	1.79254785	0.00465004	1.92087014	0.00359162	3.55572749	1.2378E-20		
ECOL4764K	85852566	<i>yeeN</i>	putative transcriptional regulator YeeN	Function Unknown (K)	1.56735099	0.18151996	1.87188402	0.08396312	1.94557903	0.01514422	1.26820165	0.9998186		
ECOL4764K	85851949		protein of unknown function	Function Unknown (S)	1.18936804	0.64643806	0.95973201	0.3662016	1.05386371	0.04779514	0.54491486	0.9998186		
ECOL4764K	85852023		conserved protein of unknown function	Function Unknown (S)	1.06079641	0.72325589	1.91186226	0.12351174	1.61571376	0.03232358	0.69774954	0.9998186		
ECOL4764K	85852530	<i>yeeX</i>	DUF496 domain-containing protein YeeX	Function Unknown (S)	1.48446556	0.12053696	1.32006713	0.00832163	1.55265854	0.00204096	1.26712195	0.42012273		
ECOL4764K	85853781		SIR_2 domain-containing protein	Function unknown (S)	1.78235876	0.05216601	2.05694257	0.00025802	2.08265483	3.475E-05	1.69739614	0.9998186		
ECOL4764K	85854633	<i>yjz2</i>	uncharacterized protein Yjz2	Function unknown (S)	1.01765908	0.99975668	0.54035335	0.99726782	0.85744316	0.39623688	2.44202815	0.00029627		
ECOL4764K	85854701		Yjbl protein	Function unknown (S)	-0.9133541	0.99975668	2.28376821	0.0383436	2.27538051	0.05809278	0.78170087	0.9998186		
ECOL4764K	85854863	<i>ybdZ</i>	enterobactin biosynthesis protein YbdZ	Function unknown (S)	-2.29213602	0.99975668	1.13394637	0.02112293	1.40956129	0.22320309	0.911427	0.9998186		
ECOL4764K	85851865	<i>stfA</i>	sorbitol-specific PTS enzyme IIC2 component	Metabolism (G)	0.45129968	0.99975668	1.62365045	0.99726782	1.2762467	0.03441003	1.14754227	0.811379		
ECOL4764K	85852338	<i>napD</i>	NapA signal peptide-binding chaperone	Metabolism (P)	0.8201188	0.99975668	2.29289548	0.05600281	2.87501484	0.00812277	2.25275005	0.06791626		
ECOL4764K	85853341	<i>narK</i>	nitratrite antipporter NarK	Metabolism (P)	-0.38390016	0.99975668	1.05699721	0.9733515	2.61825819	0.04321861	1.92704218	0.78516139		
ECOL4764K	85854703	<i>lamB</i>	maltose outer membrane channel/phage lambda receptor protein	Metabolism (M)	-0.33199188	0.99975668	3.02915833	0.03103617	2.85400101	0.01821663	1.67912896	0.9998186		
ECOL4764K	85854704	<i>malK</i>	maltose ABC transporter ATP binding subunit	Metabolism (P)	-0.91271454	0.99975668	2.91750617	0.00246239	2.87200557	0.0036916	1.26124927	0.01393791		
ECOL4764K	85854705	<i>malE</i>	maltose ABC transporter periplasmic binding protein	Metabolism (P)	-0.07773947	0.99975668	2.29063854	0.05361344	3.22362222	0.03578512	1.12427349	0.0616188		
ECOL4764K	85854706	<i>malF</i>	maltose ABC transporter membrane subunit MalF	Metabolism (G)	0.13807123	0.99975668	2.87308868	0.00955001	2.83601098	0.01086782	1.03170565	0.41672289		
ECOL4764K	85853531	<i>gnsA</i>	putative phosphatidylethanolamine synthetase, penicillinase	Metabolism (S)	1.9499088	0.01334079	1.62445128	0.56803068	1.48440702	0.44180191	0.75222366	0.9998186		
ECOL4764K	85852618	<i>fliS</i>	flagellar biosynthesis protein FliS	Mobility (N)	-0.3836078	0.99975668	1.10600922	0.47705249	1.05056155	0.31125954	1.59581178	0.00035853		
ECOL4764K	85852620	<i>fliC</i>	flagellar filament structural protein	Mobility (N)	1.27948099	0.3604938	1.36332621	0.00715182	1.47471013	0.00223327	2.60139882	6.3047E-11		
ECOL4764K	85852648	<i>motA</i>	motility protein A	Mobility (N)	2.99223387	0.01243818	2.45116842	2.1365E-06	2.67928714	5.6538E-11	4.82804021	5.9129E-22		
ECOL4764K	85852649	<i>motB</i>	motility protein B	Mobility (N)	1.88765993	0.12857638	1.62232638	0.00689759	1.54818363	0.0235335	3.54866867	6.12E-19		
ECOL4764K	85853521	<i>fliJ</i>	flagellar hook-filament junction protein 2	Mobility (N)	0.27038769	0.99975668	0.61263279	0.99726782	0.572498	0.99982751	1.70499166	1.8969E-05		
ECOL4764K	85853522	<i>fliK</i>	flagellar hook-filament junction protein 1	Mobility (N)	0.10274037	0.99975668	0.36238444	0.99726782	0.50076412	0.99982751	2.0173971	0.02232431		
ECOL4764K	85853533	<i>fliM</i>	anti-sigma factor for FliA (sigma28)	Mobility (N)	0.34000603	0.99975668	0.69973937	0.99726782	0.5804435	0.99982751	1.97572269	0.00049616		
ECOL4764K	85853534	<i>fliN</i>	flagellar biosynthesis protein FliN	Mobility (N)	-0.77326134	0.99975668	0.78099782	0.99726782	0.9081652	0.8880041	1.78599881	0.01350632		
ECOL4764K	85854634	<i>rcfC</i>	clamp-binding sister replication fork colocalization protein	Mobility/Biofilm (S)	1.7097451	0.07723678	1.95494295	0.00099113	2.21853205	0.00020283	4.27686078	4.6418E-07		
ECOL4764K	85851287	<i>pdeH</i>	c-di-GMP phosphodiesterase PdeH	Signal transduction/ Biofilm	1.46385624	0.99975668	1.66923588	0.00445625	1.71644685	0.00093775	3.34376659	9.1986E-09		
ECOL4764K	85851559	<i>oer</i>	aerotaxis sensor receptor, flavoprotein regulator of acid resistance, influenced by indole	Stress response (T)	0.7750516	0.99975668	0.55372989	0.99726782	0.57298795	0.99982751	2.01637057	7.1499E-08		
ECOL4764K	85853407	<i>anrR</i>	DNA-binding transcriptional dual regulator SoxS	Stress response (K)	-1.49354048	0.99975668	2.80049268	0.04360618	1.93625417	0.0816273	0.09458701	0.9998186		
ECOL4764K	85854675	<i>soxS</i>	DUF1471 domain-containing multiple stress resistance outer membrane protein	Stress response (M)	0.87424041	0.99975668	2.21650721	0.00259711	2.98162412	1.9613E-08	0.28148909	0.9998186		
ECOL4764K	85853493	<i>bhsA</i>	DUF1471 domain-containing stress-induced protein YbcN	Stress response (S)	1.59776978	0.2905904	2.42131465	0.00118746	2.25488823	2.7044E-06	0.96289078	0.9998186		
ECOL4764K	85853319	<i>yhcW</i>	cold shock protein CspA	Transcription (K)	1.87275648	0.07206742	3.39817662	0.00145485	2.98627883	0.00046437	1.27996133	0.9998186		
ECOL4764K	85851256	<i>capA</i>	translation initiation factor IF-1	Translation (J)	1.12139443	0.61924355	1.5684485	0.00214343	2.0167162	8.7657E-06	1.01535241	0.9998186		
ECOL4764K	85853716	<i>infA</i>	Phage protein	Phage related gene	3.14455603	0.00149673	2.18084744	0.00086307	2.52625482	0.00024066	0.9660949	0.9998186		
ECOL4764K	85854613	<i>ghoT</i>	toxin of the GhoT toxin-antitoxin system	Virulence (S)	0.83692078	0.99975668	1.12525596	0.57856784	1.84114319	0.01535673	1.14237385	0.9998186		
ECOL4764K	85853786		AAA family ATPase		1.18850346	0.64643806	1.3958336	0.06825492	1.4229527	0.03606433	0.93340617	0.9998186		
ECOL4764K	85853146	<i>ydcI</i>	putative DNA-binding transcriptional repressor YdcI	Transcription (K)	-2.69473373	0.01855179	-1.07572986	0.99726782	-1.21206268	0.57141967	-0.93255822	0.9998186		
ECOL4764K	85852863	<i>lpp</i>	murein lipoprotein	Stress response (M)	-1.7994587	0.26242052	-3.3785418	0.00014215	-2.13192355	0.27055292	-2.82669822	0.00395717		
ECOL4764K	85854468	<i>fimA</i>	type 1 fimbriae major subunit	Adherence, virulence (NU)	-0.17459055	0.99975668	-1.37091961	0.02693564	-1.26245507	0.04858227	-1.17411151	0.05009408		
ECOL4764K	85853794		conserved protein of unknown function	Function unknown (S)	-2.65112498	0.99975668	-3.08426947	0.030812	-2.78481638	0.03064935	-1.09159107	0.9998186		
ECOL4764K	85854469		conserved protein of unknown function	Function unknown (S)	-0.88397112	0.99975668	-1.59301313	0.0223801	-1.35985068	0.05618409	-1.08447934	0.9998186		
ECOL4764K	85855200	<i>cyuP</i>	putative D/L-serine transporter	Metabolism (E)	-2.46467882	0.00398487	-0.45755875	0.99726782	-0.01010979	0.99982751	0.09586933	0.9998186		
ECOL4764K	85855214	<i>garP</i>	galactarate/glycerate/glycerate ligase,garP	Metabolism (P)	-3.28602035	0.99975668	-3.2158808	0.03321983	-2.19102686	0.40414648	-1.21174616	0.9998186		
ECOL4764K	85853531	<i>flgB</i>	flagellar basal-body rod protein FlgB	Mobility (N)	-2.43763625	0.01135224	0.29402647	0.99726782	-0.05278989	0.99982751	1.05303623	0.9998186		
ECOL4764K	85853758		Phage tail assembly protein	Phage related gene	-5.95627426	0.19007441	-2.02755971	0.05361344	-2.0384184	0.01677391	-0.63342802	0.9998186		
ECOL4764K	85853759		Major tail tube protein	Phage related gene	-3.13853242	0.02475815	-3.31806964	7.0752E-05	-3.34564453	1.4221E-05	-0.75527137	0.9998186		
ECOL4764K	85853760		Phage tail protein	Phage related gene	-3.37506196	0.01825221	-3.31178787	0.00030141	-3.14187594	0.00018979	-0.61499114	0.9998186		
ECOL4764K	85853767		Baseplate assembly protein J	Phage related gene	-2.73798699	0.64643806	-2.43688833	0.01225011	-2.04635302	0.01131369	-0.44105113	0.9998186		
ECOL4764K	85853769		Baseplate assembly protein V	Phage related gene	-5.26718785	0.40760908	-1.9514778	0.03138701	-1.72378011	0.05643756	-0.44646635	0.9998186		
ECOL4764K	85853774		putative regulatory protein	Phage related gene	-4.59052364	0.73682125	-2.43467669	0.08358337	-2.95861342	0.00027917	-0.71320757	0.9998186		
ECOL4764K	85853775		Putative membrane protein	Phage related gene	-5.75732822	0.28603413	-3.6018762							



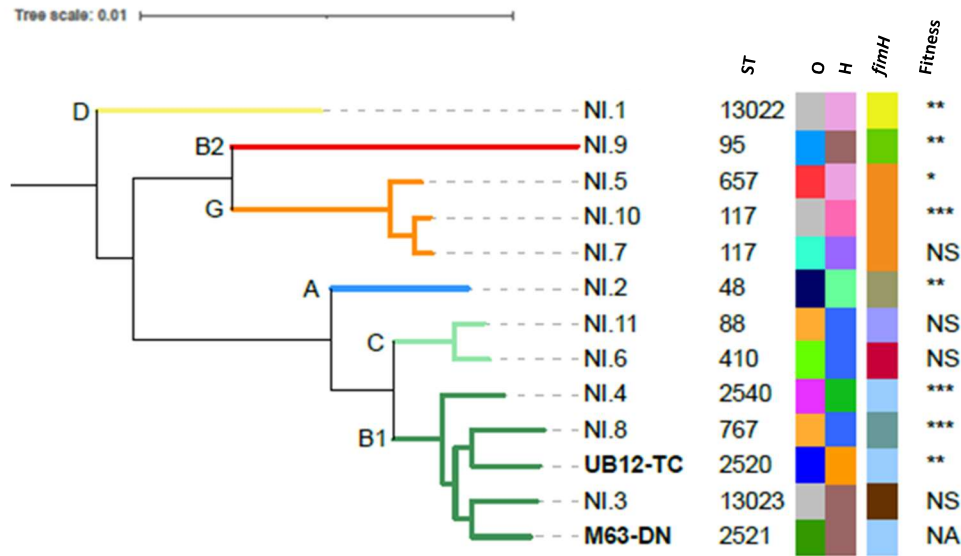
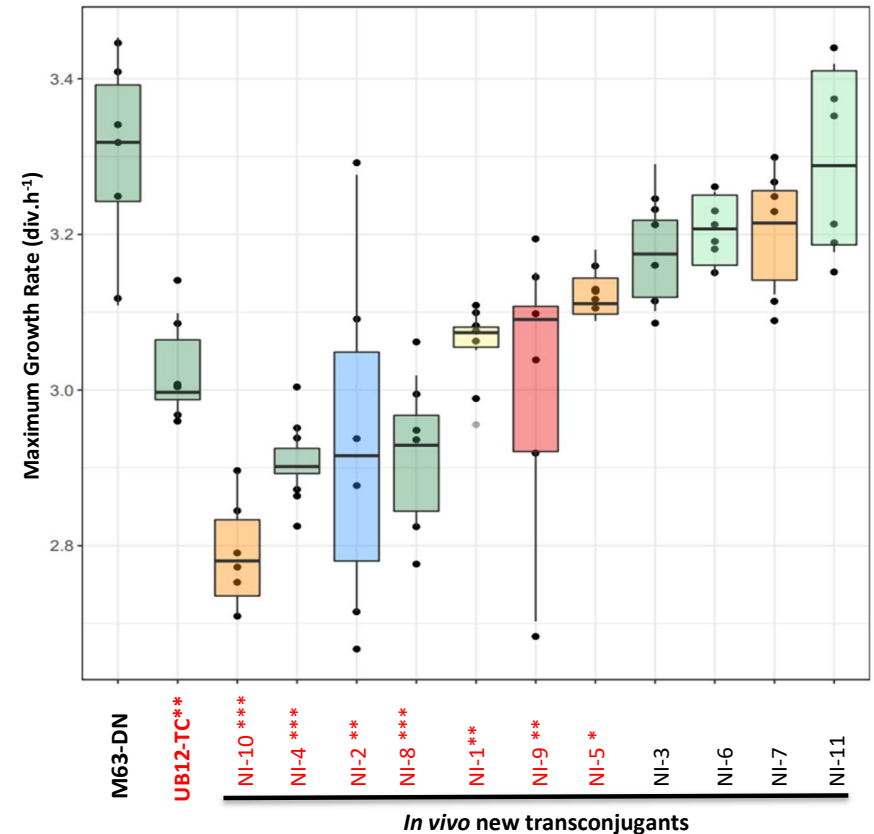


**Fig 1** : Boxplot representation of the maximum growth rate (MGR) of each pool lineage: MGR of the donor strain (M63-DN), the recipient strain (UB12-RC), the ancestral transconjugant (UB12-TC, IncI, pST3), seven *in vivo* evolved transconjugants (UB12-TC<sup>ive</sup>), and three evolved transconjugants which had lost *in vivo* the IncI plasmid (UB12-TCΔpIncl). UB12-TC<sup>ive</sup> and UB12-TCΔpIncl were isolated in stools between day 7 and day 45 during longitudinal study of *in vivo* carriage in pigs. For each box, the central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. Each point corresponds to one measurement per clone, which were repeated six times per strains. Asterisks indicate significant differences (\* =  $P < 0.05$ , \*\* =  $P < 0.01$  and \*\*\* =  $P < 0.001$ ). NS= not significant.





**Fig 2** : Boxplot representation of the maximum growth rate (MGR) of each pool lineage: MGR of five ancestral UB12-RC and UB12-TC control strains and evolved UB12-RC and UB12-TC strains at day 30. For each box, the central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. Each point corresponds to one measurement per clone, which were repeated six times per transconjugant and control. NS = not significant.

**(A)****(B)**

**FIG 3: (A)**, Phylogenetic tree of the 11 newly formed transconjugants detected among pig *E. coli* commensal isolates (NI clones, *E. coli*, pST3 IncI), the M63-DN donor strain, and the UB12-TC transconjugant strain (in bold) are presented. The tree is rooted on an *Escherichia* clade (E1492cladeI, (Denamur et al, Nat rev Microbiol, 2020)). The phylogenetic tree was constructed with IQ-TREE v1.6.9 from the core genome genes using Roary v1.007002. From left to right are presented the Sequence Type (according to the Warwick university scheme), the O and H serotypes, the *fimH* allele, and the fitness cost of NI clones compared to donor strain (M63-DN) (NA= not applicable, NS= not significant, \* =  $P < 0.05$ , \*\* =  $P < 0.01$  and \*\*\* =  $P < 0.001$ ). Each branch of the same phylogenetic group are colored (yellow, D; red, B2; orange, G; blue, A; light green, C; dark green, B1). Information on O group, H type, and *fimH* allele are given in Table 1. **(B)**, Boxplot representation of the maximum growth rate (MGR) of each strain : donor strain (M63-DN), UB12-TC ancestral transconjugant, and NI clones, isolated in stools between day 4 and day 22 during longitudinal study of *in vivo* carriage in pigs. For each box, the central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. Each point corresponds to one measurement per clone, which were repeated six times per strains. Asterisks in red indicate significant differences (\* =  $P < 0.05$ , \*\* =  $P < 0.01$  and \*\*\* =  $P < 0.001$ ). Colors correspond to the strains of the same phylogroup (color code as in A).