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1 Spread of multidrug resistance IncHI1 plasmids carrying ESBL gene *bla*_{CTX-M-1} and metabolism operon
2 of prebiotic oligosaccharides in commensal *Escherichia coli* from healthy horses, France

3

4 **Maud de Lagarde,^a Caroline Larrieu,^b Karine Praud,^b Nathalie Lallier,^b Angéline**
5 **Trotureau,^b Guillaume Sallé,^b John M. Fairbrother,^a Catherine Schouler,^b and Benoît**
6 **Doublet^{b#}**

7

8 ^aOIE Reference Laboratory for *Escherichia coli*, Faculty of Veterinary Medicine, Université
9 de Montréal, Saint-Hyacinthe, QC, Canada

10 ^bINRAE, Université de Tours, ISP, Nouzilly, France

11

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14

15 #Address correspondence to Benoît Doublet, benoit.doublet@inrae.fr

16

17 **Abstract**

18 The objective was to identify the genetic determinants and supports of expanded-spectrum
19 cephalosporin (ESC) resistance in commensal *Escherichia coli* from healthy horses in France
20 in 2015. Faecal samples from 744 adult horses were screened for ESC-resistant *E. coli*
21 isolates. The ESBL/AmpC resistance genes were identified using PCR and sequencing. ESC
22 phenotypes were horizontally transferred by conjugation or transformation. Plasmids carrying
23 ESBL/AmpC genes were typed by PCR-based replicon typing, restriction fragment length
24 polymorphism, and plasmid MLST. The ESC-resistant *E. coli* isolates were typed by *XbaI*
25 macrorestriction analysis. Sixteen stables out of 41 harboured at least one horse carrying
26 ESC-resistant *E. coli*. The proportion of individually tested horses carrying ESC-resistant *E.*
27 *coli* was 8.5% (28/328). Fifty non-redundant ESC-resistant *E. coli* isolates showing a great
28 diversity of *XbaI* macrorestriction profiles, belonged mainly to phylogroup B1, and were
29 negative for major *E. coli* virulence genes suggesting that they are commensal isolates. ESBL
30 *bla_{CTX-M}* genes were dominant (*bla_{CTX-M-1}*, n=34; *bla_{CTX-M-2}*, n=8; *bla_{CTX-M-14}*, n=2) and
31 located on conjugative plasmids belonging to various incompatibility groups (IncHI1, IncI1,
32 IncN, IncY, or non-typeable). Among these, the multidrug-resistance IncHI1-pST9 plasmids
33 were dominant and simultaneously harboured the *bla_{CTX-M-1/2}* genes and an operon enabling
34 the metabolism of short-chain fructo-oligosaccharides (scFOS). In conclusion, commensal *E.*
35 *coli* of French horses displayed a significant distribution of IncHI1-pST9 plasmids carrying
36 both the *bla_{CTX-M-1/2}* gene and the *fos* metabolism operon. This finding highlights the risk of
37 co-selection of multidrug-resistance IncHI1 plasmids carrying ESBL gene possibly mediated
38 by the use of scFOS as prebiotic in horses.

39 **1. Introduction**

40 Extended-Spectrum β -Lactamases (ESBLs) or plasmid-mediated AmpC-producing
41 Enterobacteriaceae resistant to expanded-spectrum cephalosporins, especially *Escherichia*
42 *coli*, were initially reported in human clinical settings, but recent concern about the faecal
43 carriage among healthy humans has emerged [1]. Surveillance programs monitor
44 antimicrobial resistance of pathogenic bacteria in food-producing animals to prevent food-
45 borne human contamination [2]. However, faecal carriage of ESBL/AmpC-producing
46 Enterobacteriaceae by healthy animals, especially among companion animals and horses,
47 have been under less scrutiny. Zoonotic transmission of ESBL/AmpC-producing
48 microorganisms between livestock/companion animals and humans is currently a subject of
49 intense debate [1-3]. Horses have a peculiar status being considered as companion animals,
50 working animals, or livestock depending on the circumstances. Previous studies on the
51 presence of ESBL/AmpC-producing Enterobacteriaceae in equids focused on clinical isolates,
52 the risk of horse-to-horse nosocomial spread in equine clinic, or faecal shedding after ESC
53 treatments [3-10]. Moreover, close contact with horse represents a risk factor of ESBL-
54 carriage in humans [11], although evidence of transmission of ESBL/AmpC-producing
55 isolates between healthy horses or with humans remains scarce [3-12].

56 Conjugative plasmids of incompatibility groups (Inc) A/C, F, HI, I1, L and N play a major
57 role for horizontal transfer of antimicrobial resistance genes in Enterobacteriaceae, leading to
58 the widespread diffusion of CTX-M ESBL, AmpC cephalosporinase, carbapenemase
59 resistance genes among others [13]. IncHI1 plasmids carrying the *bla*_{CTX-M-1/2} genes have been
60 described in clinical *E. coli* isolates from diseased or hospitalized horses in Belgium, the
61 Czech Republic, the Netherlands, Denmark, Germany, Sweden and France [3,4,6-8,10].
62 Moreover, complete sequences of IncHI1 type 2 plasmids (pMLST sequence type 9) carrying
63 *bla*_{CTX-M-1} in equine *E. coli* revealed that they contained the *fos* operon involved in short-chain

64 fructo-oligosaccharides (scFOS) metabolism [14]. This operon has been shown to increase the
65 colonization abilities of avian pathogenic *E. coli* in the chicken digestive tract [15].

66 We have recently reported the prevalence of, and risk factors for, fecal carriage of
67 ESBL/AmpC producing *E. coli*, at the premises level in the healthy equine population in
68 France [16]. The objective of the present study was to characterize ESBL/AmpC producing *E.*
69 *coli* shedded by healthy horses at the strain- and plasmid- level to understand the
70 ESBL/AmpC spread among horses.

71

72 **2. Materials and methods**

73 *2.1. Sampling, bacterial isolates, and antimicrobial susceptibility testing*

74 Sampling was carried out as described previously [16]. Briefly, forty-one equine facilities
75 including 21 breeding and 20 riding centres in the 4 main French administrative regions of
76 horse breeding (Normandy, Pays-de-Loire, Aquitaine, and Auvergne-Burgundy). were
77 selected on a voluntary basis. Seven hundred and forty-four healthy horses were sampled
78 during the summer of 2015. For each facility, 8 individual samples were analysed and 6 to 10
79 additional samples were pooled and processed to increase the detection of ESC-resistant
80 isolates in each facility. ESC-resistant *E. coli* isolates were selected on MacConkey agar
81 plates supplemented with 1 mg/L ceftriaxone. A set of non-redundant ESC-resistant *E. coli*
82 isolates were selected based on *Xba*I-PFGE typing (to avoid clonal isolates with identical
83 pulsotype from the same horse) for further phenotypic and genotypic analysis of antimicrobial
84 resistance. Susceptibility to 30 antibiotics and the production of ESBLs were determined by
85 the disk diffusion method on Mueller-Hinton agar and using the double-disk synergy test,
86 respectively, as recommended by EUCAST 2016 (<http://www.eucast.org/>) using disks
87 (Biorad, Marne-la-Coquette, France) containing the following antibiotics: streptomycin,
88 spectinomycin, gentamicin, kanamycin, amikacin, nalidixic acid, flumequine, ciprofloxacin,
89 enrofloxacin, chloramphenicol, florfenicol, tetracycline, sulphonamides, trimethoprim,
90 amoxicillin, amoxicillin/clavulanic acid, piperacillin, piperacillin/tazobactam, ticarcillin,
91 ticarcillin/clavulanic acid, cefalotin, cefoxitin, cefuroxime, cefoperazone, ceftriaxone,
92 ceftazidime, ceftiofur, cefepime, aztreonam and imipenem, and the *E. coli* control strain
93 ATCC25922, as previously described [4].

94

95 *2.2. Molecular typing, and phylogenetic analysis*

96 Redundancy of ESC-resistant *E. coli* isolates were investigated by *Xba*I-PFGE using the
97 Pulsenet protocol ([https://www.cdc.gov/pulsenet/PDF/ecoli-shigella-salmonella-pfge-](https://www.cdc.gov/pulsenet/PDF/ecoli-shigella-salmonella-pfge-protocol-508c.pdf)
98 [protocol-508c.pdf](https://www.cdc.gov/pulsenet/PDF/ecoli-shigella-salmonella-pfge-protocol-508c.pdf)). Electrophoresis was carried out at 14°C and 6V/cm in a BioRad CHEF-
99 DRIII system (Biorad, Marne-la-Coquette, France). Cluster analysis was done using Dice
100 similarity indices with BioNumerics 7.6 software (1 % tolerance, 1 % optimization) (Applied
101 Maths, Ghent, Belgium). Unweighted pair-group method using arithmetic (UPGMA)
102 averages were used to generate an additive tree. *E. coli* isolates were classified into the 4 main
103 ECOR phylogenetic groups by triplex PCR (*chuA*, *yjaA*, and TspE4C2) as described by
104 Clermont et al. [17]. Isolates were assigned to phylogenetic groups A, B1, B2, or D.

105

106 2.3. Detection, plasmid typing, sequencing and transferability of ESBL/AmpC genes

107 PCR assays were performed to assess the presence of ESBL/AmpC genes (*bla*_{TEM}, *bla*_{SHV},
108 *bla*_{CTX-M}, *bla*_{VEB}, *bla*_{DHA}, and *bla*_{CMY}) in the parental ESC-resistant *E. coli* isolates and in
109 transconjugants or transformants, as previously described [18]. All PCR products obtained by
110 amplification from parental isolates were sequenced by Genewiz® Europe (Takeley, UK).
111 Sequence results were compared with those registered in databases using BLAST.

112 Conjugative mating experiments were carried out using sodium azide or rifampicin resistant
113 *E. coli* K-12 J5-3 (F- *proB22 metF63*) as recipient strain, as previously described [18].
114 Transconjugants were selected on Mac Conkey agar medium supplemented with ceftriaxone
115 (1 mg/L) and sodium azide (500 mg/L) or rifampicin (250 mg/L). For unsuccessful
116 conjugative transfer, the entire plasmid content of parental ESC-resistant *E. coli* isolates was
117 purified using plasmid DNA mini kit (Qiagen, Hilden, Germany) and electroporated into *E.*
118 *coli* TOP10 (Life Technologies, Saint Aubin, France) as recipient strain. ESC-resistant *E. coli*
119 transformants were selected using ceftriaxone at 1 mg/L. ESC resistance-conferring plasmids
120 were extracted from transconjugants/transformants using Macherey–Nagel NucleoBond Xtra

121 Midi plasmid purification kit (Hoerd, France) following the manufacturer's recommendations
122 for high molecular weight plasmids. Plasmid incompatibility groups were determined using
123 the PCR-based replicon typing (PBRT) method [19,20]. Subtyping of IncHI1 and IncII
124 plasmids was performed using plasmid MLST (pMLST) [21,22]. IncHI1 Plasmids were
125 further compared using restriction fragment length polymorphism (RFLP) analysis with
126 *EcoRI*.

127

128 ***Metabolism of scFOSs conferred by ESBL/AmpC plasmids and detection of fos operon***

129 The growth ability conferred by ESBL/AmpC plasmids using scFOS as the sole source of
130 carbon was assessed in M9 minimal medium supplemented with 0.5% scFOS (Profeed P95;
131 Beghin Meiji, France) and 0.01 mg/ml L-proline (Fisher) and 0.01 mg/ml L-methionine
132 (Fisher) or 0.01 mg/ml L-leucine (Fisher) for *E. coli* K-12 J5-3 or TOP10 derivatives,
133 respectively, as previously described [15]. Transconjugants/transformants containing
134 ESBL/AmpC plasmids were grown overnight at 37°C. *E. coli* isolate BEN2908 containing the
135 chromosomally-integrated *fos* operon and plasmid-free recipient strains were used as positive
136 and negative control strains, respectively. M9 minimal medium supplemented with 0.2%
137 Casamino Acids and either 0.2% glucose (Sigma) or without carbon source was used as a
138 positive and negative control, respectively. The presence of *fos* genes was detected on plasmid
139 DNA extracted from transconjugants/transformants, by PCR as previously described [15].

140

141 3. Results and discussion

142 ESBL/AmpC-producing clinical *E. coli* isolates have been described in diseased or
143 hospitalized horses in various countries in Europe [3-8,10]. Previously, we estimated the
144 prevalence of faecal carriage of ESC-resistant isolates in healthy horses in France [16].
145 Sixteen out of 41 equine facilities (39%) harboured at least one horse carrying ESC-resistant
146 *E. coli* isolates. Among 328 healthy adult horses individually analysed, the faecal carriage rate
147 of ESC-resistant *E. coli* was 8.5% (28/328). Similar results have been reported from healthy
148 horses sampled in 2008-2009 in the UK, whereas higher proportions were found in
149 hospitalized horses in different studies [3,7,23]. This occurrence of ESC-resistant *E. coli*
150 carriage at the horse level was similar to the proportion of ESC resistance of pathogenic *E.*
151 *coli* reported in the annual report 2016 of the French surveillance network
152 (<https://www.resapath.anses.fr/>). Interestingly, the antimicrobial therapy history did not reveal
153 any treatment in the last 3 months before sampling for these 328 horses except one treated
154 with penicillin for 5 days in this period. As described in the previous paper, the screening of
155 pooled samples from 416 additional healthy horses permitted us to increase the bacterial
156 collection to 50 non-redundant ESC-resistant *E. coli* isolates.

157

158 3.1. Phylogenetic characteristics of isolates

159 Most of the isolates belonged to phylogenetic group B1 (66%, n= 33/50), followed by group
160 A (24%, n= 12/50) and group D (10%, n= 5/50). Moreover, none of the major *E. coli*
161 virulence genes in livestock animals (*eae*, *stxA*, *stx2A*, *iutA*, *eltB*, *estA*, *estB*) were found [16],
162 suggesting that they are commensal isolates. *E. coli* belonging to phylogenetic group B1 are
163 commonly described as being commensal in herbivores [24]. The present phylogroup
164 distribution is similar to that recently described by Lupu *et al.* for clinical ESBL-producing *E.*
165 *coli* from horses in France and Sweden [8]. Macrorestriction analysis by *Xba*I-PFGE showed

166 high genomic diversity (with a Dice similarity coefficient of < 80%) (Fig. 1). Nevertheless, 3
167 couples of ESC-resistant *E. coli* isolates shared the same (IDs AQC2-10-2 and AQE1-15-5) or
168 related (IDs AQC2-10-1 and AQE1-6-R; AQC2-10-3 and AQE1-15-1) macrorestriction
169 patterns, although they were isolated from distinct horses in different facilities. This suggested
170 that clonal dissemination of ESC-resistant *E. coli* may occur between horses. These different
171 breeding and riding centres were geographically close (in the same region, Aquitaine),
172 however no information on exchange or common equine competition was available to
173 establish a link between these horses. MLST analysis in recent studies of clinical ESBL-
174 producing *E. coli* from horses have shown less diversity with an overwhelming contribution
175 of major clonal complexes (CC-10, -641, -1250) spreading within equine clinics or at the
176 country level [7-9].

177

178 *3.2 Antimicrobial resistance, ESBL/AmpC genes, transferability, and plasmid typing*

179 Among these ESC-resistant *E. coli* isolates, the ESBL gene *bla*_{CTX-M-1} was predominant
180 (n=34) followed by *bla*_{CTX-M-2} (n=8) (Table 1 and Fig. 1). In addition, *bla*_{CTX-M-14} and *bla*_{SHV-}
181 ₁₂ were identified in two and five isolates, respectively. Only one isolate harboured the AmpC
182 cephalosporinase gene *bla*_{CMY-2}. Overall, these 50 commensal *E. coli* isolates were multidrug-
183 resistant (MDR) with different additional non- β -lactam resistance against aminoglycosides,
184 sulphonamides, trimethoprim, tetracycline and quinolones, except for 4 isolates resistant only
185 to β -lactams (Table 1).

186 Forty-four isolates out of 50 were able to transfer their ESC-resistance phenotype to the *E.*
187 *coli* recipient strains by conjugation. The conjugative transfer of the ESBL/AmpC genes
188 *bla*_{CTX-M-1} (28/34), *bla*_{CTX-M-2} (8/8), *bla*_{CTX-M-14} (2/2), *bla*_{SHV-12} (5/5) and *bla*_{CMY-2} (1/1) was
189 confirmed by PCR and sequencing in all transconjugants. The entire plasmid content of the
190 seven isolates that were unable to conjugate their ESC phenotype were electroporated into *E.*

191 *coli* TOP10. ESBL-producing transformants positive for *bla*_{CTX-M-1} were obtained for all
192 parental isolates (Table 1). Co-transfer of additional non- β -lactam resistance phenotypes
193 occurred, depending both on the ESBL/AmpC genes and the parental isolates (Table 1).
194 Plasmid replicon typing indicated that the *bla*_{CTX-M-1} and *bla*_{CTX-M-2} genes were mainly located
195 on conjugative IncHI1 plasmids (24/30) (Table 1). *Eco*RI-RFLP analysis of IncHI1 plasmids
196 showed 6 clusters of identical restriction profiles suggesting horizontal transmission events of
197 the same plasmid at three different scales (Fig. 2). Firstly, horizontal conjugative transfer of
198 the IncHI1-pST2 plasmid carrying *bla*_{CTX-M-1} was observed in different genetically-unrelated
199 commensal *E. coli* isolates in the intestinal microbiota of horse (see horse ID NC5-9 and
200 isolates NC5-9-B vs NC5-9-1 and NC5-9-R in Figs 1 and 2). Secondly, an IncHI1-pST9
201 plasmid carrying *bla*_{CTX-M-1} was observed in phylogenetically-unrelated commensal *E. coli*
202 isolates in different horses from the same facility or in horses from different facilities,
203 indicating cross-contamination between horses (Figs 1 and 2). Finally, slightly different
204 restriction profiles and antimicrobial resistance phenotypes were sometimes observed (see
205 NE5-19 isolates, Figs 1 and 2, table 1) suggesting the possible short-term evolution of the
206 IncHI1-pST9 plasmid carrying *bla*_{CTX-M-1} within the host, probably through antimicrobial
207 resistance gene acquisition or loss. PCR mapping of IncHI1 plasmids confirmed the
208 previously described presence of IS26-composite transposon and ISCR1-class 1 integron
209 structures carrying the *bla*_{CTX-M-1} and *bla*_{CTX-M-2} genes, respectively (data not shown) [4,14].
210 Sporadic occurrences of conjugative plasmids carrying *bla*_{CTX-M} genes and belonging to other
211 Inc groups, e.g. IncI1-pST3 and -pST87, and IncY were found in different equine facilities. In
212 addition, four IncN-pST1 plasmids carrying *bla*_{CTX-M-1} were identified in different horses
213 from the same breeding stable (Table 1). Also, two horses carried the ESBL gene *bla*_{SHV-12}
214 located on conjugative IncX3 plasmids as recently described from wildlife and horses [9,25].
215 These *bla*_{SHV-12}-IncX3 plasmids were harboured by several distinct *E. coli* isolates in each

216 horse gut microbiota. Of note, one horse (AQC2-10) simultaneously harboured the ESBL
217 genes *bla*_{CTX-M-1} and *bla*_{SHV-12}, located on IncY and IncX3 plasmids, respectively. Another
218 horse harboured two different isolates positive for *bla*_{CTX-M-1}, one on a IncI1-pST87 plasmid
219 and the other on a multireplicon IncHI1-pST9/Y plasmid (Table 1).

220 In agreement with recent studies on diseased horses, the ESBL gene *bla*_{CTX-M-1} was
221 predominant in the horses of our study [3,7,8,10]. CTX-M-1 producers are also predominant
222 in food-producing animals in several European countries [13]. IncHI1 plasmids carrying
223 *bla*_{CTX-M-1/2} genes have been reported in *E. coli* isolates from diseased horses in hospital
224 settings in Belgium, the Netherlands, the Czech Republic, Germany, Sweden and France
225 [3,4,6-8,10]. Nevertheless, our results demonstrate that other epidemic plasmids carrying
226 *bla*_{CTX-M-1}, i.e. IncI1-pST3 or IncN-pST1, as well as another ESBL-plasmid association
227 (*bla*_{SHV-12}-IncX3) are also harboured by commensal *E. coli* among healthy horses in France.

228

229 *3.3 bla*_{CTX-M} *IncHI1-pST9* plasmids confer the property of *scFOS* metabolism

230 The *fos* operon was initially characterized on the chromosomal genomic island AGI-3 in
231 extraintestinal avian pathogenic *E. coli* [15]. This operon is involved in the metabolism of
232 prebiotic short-chain fructooligosaccharides (scFOS) and was shown to play a major role in
233 the initial stage of chicken intestinal colonization by *E. coli* [26]. Recently, Dolejska *et al.*
234 described the presence of the functional *fos* operon in IncHI1-pST9 plasmids pEQ in *E. coli*
235 from diseased horses in the Czech Republic [14]. In the present study, the *fos* operon was
236 detected by PCR in all except one *bla*_{CTX-M1/2}-IncHI1-pST9 plasmids (Table 1). All *E. coli*
237 transconjugants/transformants carrying *fos*-positive IncHI1-pST9 plasmids were able to grow
238 in M9 minimal medium supplemented with scFOS as the sole carbon source (Table 1),
239 which was not the case for the different empty recipient *E. coli* strains corresponding to
240 negative controls. This plasmid-encoded metabolic function may constitute an advantage for

241 colonization of the intestinal microbiota of horses. Moreover, the use of scFOS as prebiotic
242 additive in feed or as a treatment of digestive disorders for horses may also contribute to co-
243 selection and maintenance of MDR IncHI1 plasmids in the absence of antibiotic selection
244 pressure. However, this hypothesis warrants further investigations in controlled experimental
245 settings or a cross-sectional study.

246

247 In conclusion, a significant occurrence of ESBL-producing *E. coli* was found in healthy
248 horses in France. A large diversity of phylogenetic backgrounds of commensal *E. coli* was
249 found but carrying specific *bla*_{CTX-M} plasmids such as IncHI1-pST9 plasmids carrying both
250 the ESBL genes *bla*_{CTX-M1/2} and the *fos* operon, which is disseminated across Europe. Here,
251 different examples argue on the horizontal conjugative transfer of these plasmids *in vivo*
252 within the horse gut microbiota. Other plasmid-encoded functions than antimicrobial
253 resistance such as scFOS metabolism may represent determinants for the spread of IncHI1
254 plasmids carrying *bla*_{CTX-M-1} in horses. However, further investigations are needed to confirm
255 their co-selection and maintenance through the use of scFOS. Overall, ESBL dissemination
256 among horses in Europe and potentially worldwide is most likely facilitated by international
257 horse movements associated with racing, breeding activities and hospitalization. Horses
258 should be considered as a potential reservoir of ESBL/AmpC resistance genes. Thus, further
259 surveillance of antimicrobial resistance in the equine environment is necessary to reduce the
260 dissemination of critically-important resistances and to investigate the public health risks.

261

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270 **Ethical Approval:** Not required

271

272 **References**

- 273 [1] Woerther PL, Burdet C, Chachaty E, Andremont A. 2013. Trends in human fecal
274 carriage of extended-spectrum β -lactamases. *Clin Microbiol Rev* 26:744-758.
- 275 [2] EFSA. 2017. The European Union summary report on antimicrobial resistance in
276 zoonotic and indicator bacteria from humans, animals and food in 2015. *EFSA J* 15:4694.
- 277 [3] Dolejska M, Duskova E, Rybarikova J, Janoszowska D, Roubalova E, Dibdakova K,
278 Maceckova G, Kohoutova L, Literak I, Smola J, Cizek A. 2011. Plasmids carrying *bla*_{CTX-M-1}
279 and *qnr* genes in *Escherichia coli* isolates from an equine clinic and a horseback riding centre.
280 *J Antimicrob Chemother* 66:757-764.
- 281 [4] Smet A, Boyen F, Flahou B, Doublet B, Praud K, Martens A, Butaye P, Cloeckeaert A,
282 Haesebrouck F. 2012. Emergence of CTX-M-2-producing *Escherichia coli* in diseased
283 horses: evidence of genetic exchanges of *bla*_{CTX-M-2} linked to *ISCR1*. *J Antimicrob Chemother*
284 67:1289-1291.
- 285 [5] Damborg P, Marskar P, Baptiste KE, Guardabassi L. 2012. Faecal shedding of CTX-
286 M-producing *Escherichia coli* in horses receiving broad-spectrum antimicrobial prophylaxis
287 after hospital admission. *Vet Microbiol* 154:298-304.
- 288 [6] Jakobsen L, Bortolaia V, Bielak E, Moodley A, Olsen SS, Hansen D, Frimodt-Møller,
289 Guardabassi L, Hasman H. 2015. Limited similarity between plasmids encoding CTX-M-1 β -
290 lactamase in *Escherichia coli* from humans, pigs, cattle, organic poultry layers and horses in
291 Denmark. *J Glob Antimicrob Resist* 3:132-136.
- 292 [7] Apostolakos I, Franz E, van Hoek AHAM, Florijn A, Veenman C, Sloet-van
293 Oldruitenborgh-Oosterbaan MM, Dierikx C, van Duijkeren E. 2017. Occurrence and
294 molecular characteristic of ESBL/AmpC-producing *Escherichia coli* in faecal samples from
295 horses in an equine clinic. *J Antimicrob Chemother* 72:1915-1921.

- 296 [8] Lupo A, Haenni M, Saras E, Gradin J, Madec JY, Haenni M. 2018 Is *bla*_{CTX-M-1} riding
297 the same plasmid among horses in Sweden and France? *Microb Drug Resist* 24:1580-1586.
- 298 [9] Sadikalay S, Reynaud Y, Guyomard-Rabanirima S, Falord M, Ducat C, Fabre L, Le
299 Hello S, Talarmin A, Ferdinand S. 2018. High genetic diversity of extended-spectrum β -
300 lactamases producing *Escherichia coli* in feces of horses. *Vet Microbiol* 219:117-222.
- 301 [10] Walther B, Klein KS, Barton AK, Semmler T, Huber C, Wolf SA, Tedin K, Merle R,
302 Mitrach F, Guenther S, Lübke-Becker A, Gehlen H. 2018. Extended-spectrum beta-lactamase
303 (ESBL)-producing *Escherichia coli* and *Acinetobacter baumannii* among horses entering a
304 veterinary teaching hospital: The contemporary “Trojan horse”. *PLoS ONE* 13:e0191873.
- 305 [11] Huijbers PM, de Kraker M, Graat EA, van Hoek AH, van Santen MG, de Jong MC,
306 van Duijkeren E, de Greeff SC. 2013. Prevalence of extended-spectrum beta-lactamase-
307 producing Enterobacteriaceae in humans living in municipalities with high and low broiler
308 density. *Clin Microbiol Infect* 19:E256-9.
- 309 [12] Maddox TW, Clegg PD, Williams NJ, Pinchbeck GL. 2015 Antimicrobial resistance
310 in bacteria from horses: Epidemiology of antimicrobial resistance. *Equine Vet J* 47:756-765.
- 311 [13] Rozwandowicz M, Brouwer MSM, Fischer J, Wagenaar JA, Gonzalez-Zorn B, Guerra
312 B, Mevius D, Hordijk J. 2018 Plasmids carrying antimicrobial resistance genes in
313 Enterobacteriaceae. *J Antimicrob Chemother* 73:1121-1137.
- 314 [14] Dolejska M, Villa L, Minoia M, Guardabassi L, Carattoli A. 2014. Complete
315 sequences of IncHI1 plasmids carrying *bla*_{CTX-M-1} and *qnrS1* in equine *Escherichia coli*
316 provide new insights into plasmid evolution. *J Antimicrob Chemother* 69:2388-2393.
- 317 [15] Schouler C, Taki A, Chouikha I, Moulin-Schouleur M, Gilot P. 2009. A genomic
318 island of an extraintestinal pathogenic *Escherichia coli* strain enables the metabolism of
319 fructooligosaccharides, which improves intestinal colonization. *J Bacteriol* 191:388-393.

- 320 [16] de Lagarde M, Larrieu C, Praud K, Schouler C, Doublet B, Sallé G, Fairbrother JM,
321 Arsenault J. 2019. Prevalence, risk factors, and characterization of multidrug resistant and
322 ESBL/AmpC producing *Escherichia coli* in healthy horses in France in 2015. *J Vet Int Med*
323 33:902-911.
- 324 [17] Clermont O, Bonacorsi S, Bingen E. 2000. Rapid and simple determination of the
325 *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* 66:4555-4558.
- 326 [18] Doublet B, Praud K, Nguyen-Ho-Bao T, Argudín MA, Bertrand S, Butaye P,
327 Cloeckaert A. 2014. Extended-spectrum β -lactamase- and AmpC β -lactamase-producing D-
328 tartrate-positive *Salmonella enterica* serovar Paratyphi B from broilers and human patients in
329 Belgium, 2008–10. *J Antimicrob Chemother* 69:1257-1264.
- 330 [19] Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. 2005.
331 Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 63:219-228.
- 332 [20] Carloni E, Adreoni F, Omiccioli E, Villa L, Magnani M, Carattoli A. 2017.
333 Comparative analysis of the standard PCR-based replicon typing (PBRT) with the commercial
334 PBRT-KIT. *Plasmid* 90:10-14.
- 335 [21] Phan MD, Kidgell C, Nair S, Holt KE, Turner AK, Hinds J, Butcher P, Cooke FJ,
336 Thomson NR, Titball R, Bhutta ZA, Hasan R, Dougan G, Wain J. 2009. Variation in
337 *Salmonella enterica* serovar Typhi IncHI1 plasmids during the global spread of resistant
338 typhoid fever. *Antimicrob Agents Chemother* 53:716-727.
- 339 [22] Cain AK, Hall RM. 2013. Evolution of IncHI1 plasmids: Two distinct lineages.
340 *Plasmid* 70:201-208.
- 341 [23] Maddox TW, Clegg PD, Diggle PJ, Wedley AL, Dawson S, Pinchbeck GL, Williams
342 NJ. 2012. Cross-sectional study of antimicrobial-resistant bacteria in horses. Part 1:
343 Prevalence of antimicrobial-resistant *Escherichia coli* and methicillin-resistant
344 *Staphylococcus aureus*. *Equine Vet J* 44:289-296.

- 345 [24] Tenailon O, Skurnik D, Picard B, Denamur E. 2010. The population genetics of
346 commensal *Escherichia coli*. Nat Rev Microbiol 8:207-217.
- 347 [25] Dobiasova H, Dolejska M. 2016. Prevalence and diversity of IncX plasmids carrying
348 fluoroquinolone and β -lactam resistance genes in *Escherichia coli* originating from diverse
349 sources and geographical areas. J Antimicrob Chemother 71: 2118-2124.
- 350 [26] Porcheron G, Kut E, Canepa S, Maurel MC, Schouler C. 2011. Regulation of
351 fructooligosaccharide metabolism in an extra-intestinal pathogenic *Escherichia coli* strain.
352 Mol Microbiol 81:717-733.

353

354 **Figure legends:**

355 **Fig. 1.** Analysis of *Xba*I-PFGE patterns obtained from ESBL/Amp^C-producing *E. coli*
356 isolates.

357 PFGE profiles were compared by using BioNumerics software version 7.6 (Applied Maths)
358 with settings of 1.0% optimization and 1.0% tolerance. Isolate ID contains all information
359 relative to the origin of the isolates as follows XX(E/C)n-n-XX: The first letter or two first
360 letters correspond to the French administrative regions (AB, Auvergne-Burgundy; AQ,
361 Aquitaine; N, Normandy; PL, Pays-de-Loire). “En” or “Cn” correspond to the breeding
362 facility (E) or equestrian centre (C) number. The horse number in the facility is indicated
363 between dashes. The last letter or number is specific for the isolate, used when there are
364 several isolates from the same horse. Coloured tree branches and symbols before Isolate IDs
365 correspond to isolates from the same horse. *E. coli* isolate AQE4-4-RO could not be restricted
366 by *Xba*I-PFGE in repeated attempts, thus was excluded from the present analysis.

367

368 **Fig. 2.** Analysis of *Eco*RI-RFLP profiles of IncHI1 plasmids carrying *bla*_{CTX-M1/2} genes.

369 Coloured tree branches correspond to plasmids from the same animal (blue and green) or
370 distinct animals from the same facility as well as from different facilities (red).* IncHI1
371 plasmids carrying *bla*_{CTX-M-2}. *fos* operon presence/absence, ■/□. IncHI1-ST9 plasmid
372 pRCS78 was previously described and added as control (complete sequence available,
373 GenBank accession number LT985296) (4). DNA of IncHI1 plasmids from *E. coli* isolates
374 ABE1-40-R, AQE4-7-B, and NC0-17-R could not be restricted by *Eco*RI in repeated
375 attempts, thus was excluded from the present analysis.

376

377 **Table 1.** Characteristics of ESC-resistant *E. coli* strains from horses, France.

Isolate ID ^a	Non β -lactam resistance phenotype of parental strain	Transferred	Plasmid	Non β -lactam resistance phenotype	ScFOS growth	<i>fos</i>
		ESBL/AmpC resistance gene	replicon Inc group-pMLST ^b	transferred	conferred by ESBL/ampC plasmids	metabolism operon
ABE1-40-B	GEN STR SPT SUL TMP TET NAL FLU CIP ENR	<i>bla</i> _{CTX-M-14}	I1-pST3 (CC3)	SUL TMP	-	-
ABE1-40-R	GEN KAN STR SUL TMP TET	<i>bla</i> _{CTX-M-2}	HI1-pST9	GEN KAN STR SUL TMP TET	+	+
ABE1-42-R	GEN KAN STR SUL TMP TET	<i>bla</i> _{CTX-M-2}	HI1-pST9	KAN STR SUL TMP TET	+	+
ABE1-6-R	SUL TMP	<i>bla</i> _{CTX-M-1}	I1-pST3 (CC3)	SUL TMP	-	-
ABE1-P2-CR	GEN KAN STR SUL TMP TET	<i>bla</i> _{CTX-M-2}	HI1	GEN KAN STR SUL TMP TET	+	+
ABE1-P2-R	GEN KAN STR SUL TMP TET	<i>bla</i> _{CTX-M-2}	HI1-pST9	GEN KAN STR SUL TMP TET	+	+
ABE1-P3-R	SUL TMP	<i>bla</i> _{CTX-M-1}	I1-pST3 (CC3)	SUL TMP	-	-
AQC2-10-1	GEN STR SUL TMP	<i>bla</i> _{CTX-M-1}	Y	GEN STR SUL TMP	-	-
AQC2-10-2	FLU ENR	<i>bla</i> _{SHV-12}	X3	None	-	-
AQC2-10-3	SUL TMP NAL FLU	<i>bla</i> _{SHV-12}	X3	None	-	-
AQC2-10-4	SUL TMP FLU ENR	<i>bla</i> _{SHV-12}	X3	None	-	-
AQC2-2-R	CHL GEN KAN STR SUL TMP TET	<i>bla</i> _{CTX-M-1}	HI1*	CHL GEN STR SUL TET TMP	+	+
AQC6-2-R	None	<i>bla</i> _{CMY-2}	I1-pST2 (CC2)	None	-	-
AQC6-4-R	CHL GEN KAN STR SUL TMP TET	<i>bla</i> _{CTX-M-1}	HI1-pST9	CHL GEN KAN STR SUL TMP TET	+	+

AQC6-7-B	CHL GEN KAN STR SUL TMP TET	<i>bla</i> _{CTX-M-1}	HI1-pST9*	CHL GEN KAN STR SUL TMP TET	+	+
AQC6-8-R	CHL GEN KAN STR SUL TMP TET	<i>bla</i> _{CTX-M-1}	HI1-pST9	CHL GEN KAN STR SUL TMP TET	+	+
AQC7-7-R	CHL GEN KAN STR SUL TMP TET	<i>bla</i> _{CTX-M-1}	HI1	CHL GEN KAN STR SUL TMP TET	+	+
AQE1-15-1	SUL TMP TET NAL FLU CIP ENR	<i>bla</i> _{SHV-12}	X3	SUL TMP TET FLU	-	-
AQE1-15-5	FLU CIP ENR	<i>bla</i> _{SHV-12}	X3	None	-	-
AQE1-6-R	GEN STR SUL TMP	<i>bla</i> _{CTX-M-1}	Y	GEN STR SUL TMP	-	-
AQE1-8-R	CHL GEN KAN SUL TMP TET	<i>bla</i> _{CTX-M-1}	HI1-pST9	CHL GEN KAN STR SUL TMP TET	+	+
AQE3-1-RC	SUL TMP	<i>bla</i> _{CTX-M-1}	N-pST1	None	-	-
AQE3-2-R	None	<i>bla</i> _{CTX-M-1}	N-pST1	None	-	-
AQE3-4-R	None	<i>bla</i> _{CTX-M-1}	N-pST1	None	-	-
AQE3-5-R	None	<i>bla</i> _{CTX-M-1}	N-pST1	None	-	-
AQE3-6-R	CHL GEN STR SUL TMP TET	<i>bla</i> _{CTX-M-1}	HI1-pST9	CHL GEN STR SUL TMP TET	+	+
AQE4-4-RO	CHL GEN KAN STR SPT SUL TMP TET	<i>bla</i> _{CTX-M-1}	HI1	CHL GEN KAN STR SUL TMP TET	+	+
AQE4-7-B	CHL GEN KAN STR SPT SUL TMP TET	<i>bla</i> _{CTX-M-1}	HI1-pST9	CHL GEN KAN STR SPT SUL TMP TET	+	+
AQE7-P1-R	CHL GEN STR SPT SUL TMP TET	<i>bla</i> _{CTX-M-1}	HI1	CHL GEN STR SUL TMP TET	+	+
NC0-4-RC	CHL GEN KAN STR SUL TMP TET	<i>bla</i> _{CTX-M-1}	HI1	CHL GEN KAN STR TMP TET	+	+
NC0-17-1	KAN STR SUL TMP TET	<i>bla</i> _{CTX-M-2}	HI1-pST9/Y	KAN STR SUL TMP TET	+	+
NC0-17-R	GEN KAN STR SUL TMP TET	<i>bla</i> _{CTX-M-2}	I1-pST87	SUL TMP	-	-
NC5-9-1	CHL GEN SPT STR SUL TMP TET	<i>bla</i> _{CTX-M-1}	HI1-pST2*	CHL GEN SUL TMP TET	-	-

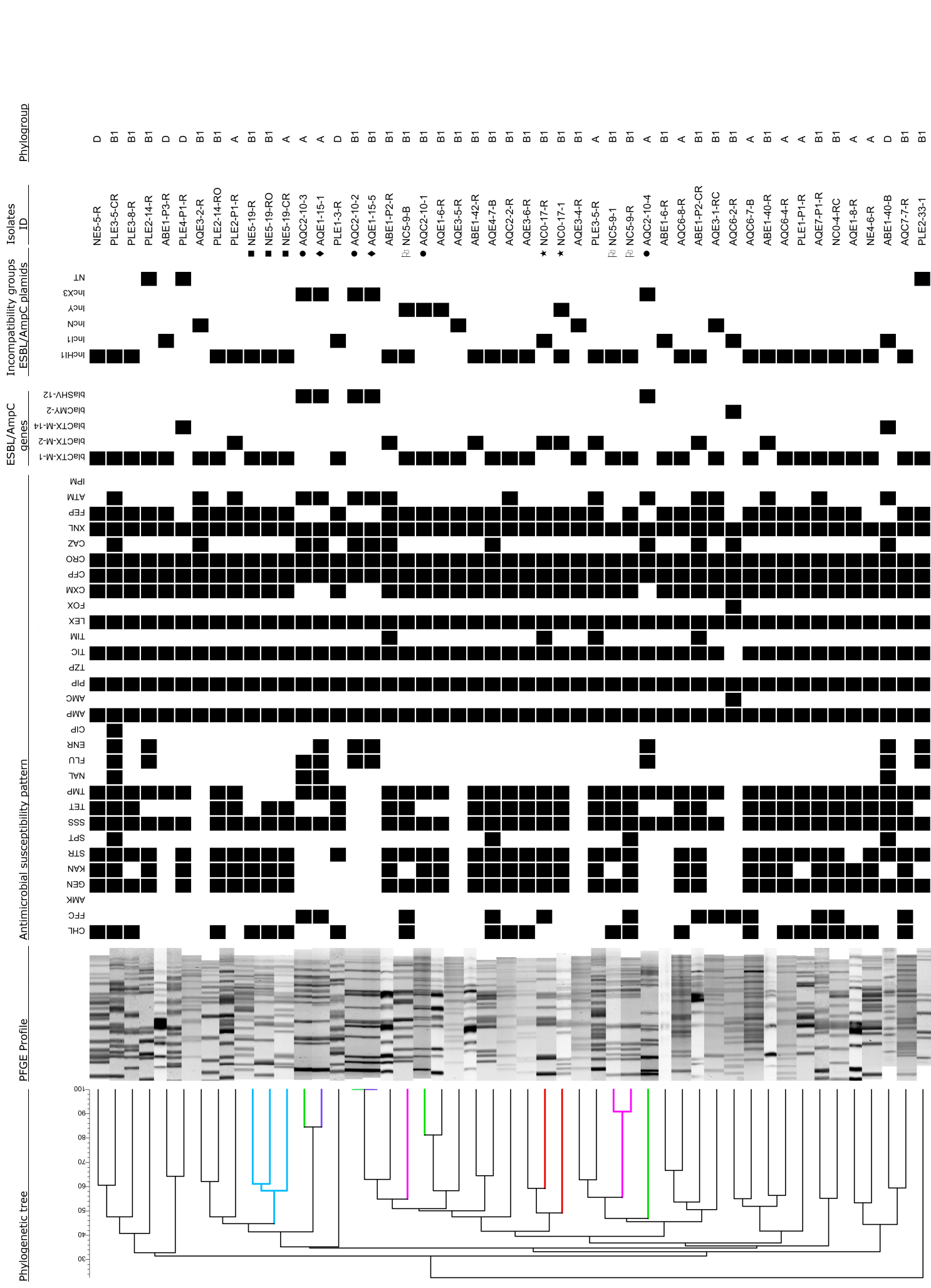
NC5-9-B	CHL GEN STR SUL TMP TET	<i>bla</i> _{CTX-M-1}	HI1-ST2	CHL STR SUL TMP TET	-	-
NC5-9-R	CHL GEN STR SPT SUL TMP TET	<i>bla</i> _{CTX-M-1}	HI1-ST2*	CHL GEN STR SUL TET TMP	-	-
NE4-6-R	CHL GEN KAN STR SUL TMP TET	<i>bla</i> _{CTX-M-1}	HI1-pST9	CHL GEN TMP	+	+
NE5-19-CR	CHL GEN KAN STR SUL TET	<i>bla</i> _{CTX-M-1}	HI1-pST9	CHL GEN KAN STR SUL TET	+	+
NE5-19-R	CHL GEN KAN STR SUL TET	<i>bla</i> _{CTX-M-1}	HI1-pST9	CHL GEN KAN STR SUL TET	+	+
NE5-19-RO	CHL GEN STR SUL TET	<i>bla</i> _{CTX-M-1}	HI1-pST9	CHL GEN STR SUL TET	+	+
NE5-5-R	CHL GEN KAN STR SUL TMP TET	<i>bla</i> _{CTX-M-1}	HI1	CHL GEN KAN STR SUL TMP TET	+	+
PLE1-3-R	CHL STR SUL TMP TET	<i>bla</i> _{CTX-M-1}	I1-pST87	SUL TMP	-	-
PLE1-P1-R	CHL GEN STR SUL TET	<i>bla</i> _{CTX-M-1}	HI1*	CHL GEN STR SUL TET TMP	+	+
PLE2-14-R	GEN STR SUL TMP FLU ENR	<i>bla</i> _{CTX-M-1}	NT*	GEN STR	-	-
PLE2-14-RO	CHL GEN STR SUL TMP TET	<i>bla</i> _{CTX-M-1}	HI1-pST9	CHL GEN STR SUL TMP TET	-	-
PLE2-33-1	GEN STR SUL TMP FLU ENR	<i>bla</i> _{CTX-M-1}	NT	GEN STR SUL TMP	+	+
PLE2-P1-R	GEN KAN STR SUL TMP TET	<i>bla</i> _{CTX-M-2}	HI1-pST9	KAN STR SUL TMP TET	+	+
PLE3-5-CR	CHL GEN KAN STR SPT SUL TMP TET NAL CIP ENR	<i>bla</i> _{CTX-M-1}	HI1*	CHL GEN KAN STR SUL TET	+	+
PLE3-5-R	KAN STR SUL TMP TET	<i>bla</i> _{CTX-M-2}	HI1	KAN STR SUL TMP TET	+	+
PLE3-8-R	CHL GEN STR SUL TMP TET	<i>bla</i> _{CTX-M-1}	HI1-pST9	CHL GEN STR SUL TMP TET	+	+
PLE4-P1-R	GEN STR SUL TMP TET	<i>bla</i> _{CTX-M-14}	NT	GEN STR SUL TMP TET	-	-

378 ^aIsolate ID contain all information relative to the origin of the isolates as follow XX(E/C)n-n-XX: The first letter or two first letters correspond to the French
379 administrative regions (AB, Auvergne-Burgundy; AQ, Aquitaine; N, Normandy; PL, Pays-de-Loire). “En” or “Cn” correspond to the breeding facility (E) or

380 equestrian centre (C) number. The horse number in the facility is indicated between dashes. The last letter or number is specific of the isolate, used when there
381 are several isolates from the same horse.

382 ^bNT, non-typeable. Asterisks indicate non-self-conjugative plasmids.

383



Phylogenetic tree

PFGE Profile

Antimicrobial susceptibility pattern

ESBL/AmpC genes

Incompatibility groups ESBL/AmpC plasmids

Isolates ID

Phylogroup

