Spread of multidrug-resistant IncHI1 plasmids carrying ESBL gene blaCTX-M-1 and metabolism operon of prebiotic oligosaccharides in commensal Escherichia coli from healthy horses, France

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

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Abstract

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

1. Introduction

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

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

2. Materials and methods

2.1. Sampling, bacterial isolates, and antimicrobial susceptibility testing

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

2.2. Molecular typing, and phylogenetic analysis

Redundancy of ESC-resistant *E. coli* isolates were investigated by *Xba*I-PFGE using the Pulsenet protocol (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-DRIII system (Biorad, Marne-la-Coquette, France). Cluster analysis was done using Dice similarity indices with BioNumerics 7.6 software (1 % tolerance, 1 % optimization) (Applied Maths, Ghent, Belgium). Unweighted pair-group method using arithmetic (UPGMA) averages were used to generate an additive tree. *E. coli* isolates were classified into the 4 main ECOR phylogenetic groups by triplex PCR (c*huA*, *yjaA,* and TspE4C2) as described by Clermont et al. [17]. Isolates were assigned to phylogenetic groups A, B1, B2, or D.

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

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

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

Midi plasmid purification kit (Hoerdt, France) following the manufacturer's recommendations for high molecular weight plasmids. Plasmid incompatibility groups were determined using the PCR-based replicon typing (PBRT) method [19,20]. Subtyping of IncHI1 and IncI1 plasmids was performed using plasmid MLST (pMLST) [21,22]. IncHI1 Plasmids were further compared using restriction fragment length polymorphism (RFLP) analysis with *Eco*RI.

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

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

3. Results and discussion

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

3.1. Phylogenetic characteristics of isolates

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

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

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

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

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

coli TOP10. ESBL-producing transformants positive for *bla*$_{CTX-M-1}$ were obtained for all parental isolates (Table 1). Co-transfer of additional non-β-lactam resistance phenotypes occurred, depending both on the ESBL/AmpC genes and the parental isolates (Table 1). Plasmid replicon typing indicated that the *bla*$_{CTX-M-1}$ and *bla*$_{CTX-M-2}$ genes were mainly located on conjugative IncHI1 plasmids (24/30) (Table 1). *Eco*RI-RFLP analysis of IncHI1 plasmids showed 6 clusters of identical restriction profiles suggesting horizontal transmission events of the same plasmid at three different scales (Fig. 2). Firstly, horizontal conjugative transfer of the IncHI1-pST2 plasmid carrying *bla*$_{CTX-M-1}$ was observed in different genetically-unrelated commensal *E. coli* isolates in the intestinal microbiota of horse (see horse ID NC5-9 and isolates NC5-9-B vs NC5-9-1 and NC5-9-R in Fig.s 1 and 2). Secondly, an IncHI1-pST9 plasmid carrying *bla*$_{CTX-M-1}$ was observed in phylogenetically-unrelated commensal *E. coli* isolates in different horses from the same facility or in horses from different facilities, indicating cross-contamination between horses (Figs 1 and 2). Finally, slightly different restriction profiles and antimicrobial resistance phenotypes were sometimes observed (see NE5-19 isolates, Figs 1 and 2, table 1) suggesting the possible short-term evolution of the IncHI1-pST9 plasmid carrying *bla*$_{CTX-M-1}$ within the host, probably through antimicrobial resistance gene acquisition or loss. PCR mapping of IncHI1 plasmids confirmed the previously described presence of IS*26*-composite transposon and IS*CR1*-class 1 integron structures carrying the *bla*$_{CTX-M-1}$ and *bla*$_{CTX-M-2}$ genes, respectively (data not shown) [4,14].

Sporadic occurrences of conjugative plasmids carrying *bla*$_{CTX-M}$ genes and belonging to other Inc groups, e.g. IncI1-pST3 and -pST87, and IncY were found in different equine facilities. In addition, four IncN-pST1 plasmids carrying *bla*$_{CTX-M-1}$ were identified in different horses from the same breeding stable (Table 1). Also, two horses carried the ESBL gene *bla*$_{SHV-12}$ located on conjugative IncX3 plasmids as recently described from wildlife and horses [9,25]. These *bla*$_{SHV-12}$-IncX3 plasmids were harboured by several distinct *E. coli* isolates in each

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

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

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

The *fos* operon was initially characterized on the chromosomal genomic island AGI-3 in extraintestinal avian pathogenic *E. coli* [15]. This operon is involved in the metabolism of prebiotic short-chain fructooligosaccharides (scFOS) and was shown to play a major role in the initial stage of chicken intestinal colonization by *E. coli* [26]. Recently, Dolejska *et al.* described the presence of the functional *fos* operon in IncHI1-pST9 plasmids pEQ in *E. coli* from diseased horses in the Czech Republic [14]. In the present study, the *fos* operon was detected by PCR in all except one *bla*$_{\text{CTX-M1/2}}$-IncHI1-pST9 plasmids (Table 1). All *E. coli* transconjugants/transformants carrying *fos*-positive IncHI1-pST9 plasmids were able to grow in M9 minimal medium supplemented with scFOS as the sole carbone source (Table 1), which was not the case for the different empty recipient *E. coli* strains corresponding to 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*$_{\text{CTX-M}}$ plasmids such as IncHI1-pST9 plasmids carrying both
250 the ESBL genes *bla*$_{\text{CTX-M1/2}}$ 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*$_{\text{CTX-M-1}}$ 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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References

[1] Woerther PL, Burdet C, Chachaty E, Andremont A. 2013. Trends in human fecal carriage of extended-spectrum β-lactamases. Clin Microbiol Rev 26:744-758.

[2] EFSA. 2017. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015. EFSA J 15:4694.

[3] Dolejska M, Duskova E, Rybarikova J, Janoszowska D, Roubalova E, Dibdakova K, Maceckova G, Kohoutova L, Literak I, Smola J, Cizek A. 2011. Plasmids carrying *bla*$_{\text{CTX-M-1}}$ and *qnr* genes in *Escherichia coli* isolates from an equine clinic and a horseback riding centre. J Antimicrob Chemother 66:757-764.

[4] Smet A, Boyen F, Flahou B, Doublet B, Praud K, Martens A, Butaye P, Cloeckaert A, Haesebrouck F. 2012. Emergence of CTX-M-2-producing *Escherichia coli* in diseased horses: evidence of genetic exchanges of *bla*$_{\text{CTX-M-2}}$ linked to IS*CR1*. J Antimicrob Chemother 67:1289-1291.

[5] Damborg P, Marskar P, Baptiste KE, Guardabassi L. 2012. Faecal shedding of CTX-M-producing *Escherichia coli* in horses receiving broad-spectrum antimicrobial prophylaxis after hospital admission. Vet Mirobiol 154:298-304.

[6] Jakobsen L, Bortolaia V, Bielak E, Moodley A, Olsen SS, Hansen D, Frimodt-Møller, Guardabassi L, Hasman H. 2015. Limited similarity between plasmids encoding CTX-M-1 β-lactamase in *Escherichia coli* from humans, pigs, cattle, organic poultry layers and horses in Denmark. J Glob Antimicrob Resist **3**:132-136.

[7] Apostolakos I, Franz E, van Hoek AHAM, Florijn A, Veenman C, Sloet-van Oldruitenborgh-Oosterbaan MM, Dierikx C, van Duijkeren E. 2017. Occurrence and molecular characteristic of ESBL/AmpC-producing *Escherichia coli* in faecal samples from 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*$_{\text{CTX-M-1}}$ 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* an *Acinetobacter baumanii* 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*$_{\text{CTX-M-1}}$ 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.

[16] de Lagarde M, Larrieu C, Praud K, Schouler C, Doublet B, Sallé G, Fairbrother JM, Arsenault J. 2019.Prevalence, risk factors, and characterization of multidrug resistant and ESBL/AmpC producing *Escherichia coli* in healthy horses in France in 2015. J Vet Int Med 33:902-911.

[17] Clermont O, Bonacorsi S, Bingen E. 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol 66:4555-4558.

[18] Doublet B, Praud K, Nguyen-Ho-Bao T, Argudín MA, Bertrand S, Butaye P, Cloeckaert A. 2014. Extended-spectrum β-lactamase- and AmpC β-lactamase-producing D-tartrate-positive *Salmonella enterica* serovar Paratyphi B from broilers and human patients in Belgium, 2008–10. J Antimicrob Chemother 69:1257-1264.

[19] Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. 2005. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 63:219-228.

[20] Carloni E, Adreoni F, Omiccioli E, Villa L, Magnani M, Carattoli A. 2017. Comparative analysis of the standard PCR-based replicon typing (PBRT) with the commercial PBRT-KIT. Plasmid 90:10-14.

[21] Phan MD, Kidgell C, Nair S, Holt KE, Turner AK, Hinds J, Butcher P, Cooke FJ, Thomson NR, Titball R, Bhutta ZA, Hasan R, Dougan G, Wain J. 2009. Variation in *Salmonella enterica* serovar Typhi IncHI1 plasmids during the global spread of resistant typhoid fever. Antimicrob Agents Chemother 53:716-727.

[22] Cain AK, Hall RM. 2013. Evolution of IncHI1 plasmids: Two distinct lineages. Plasmid 70:201-208.

[23] Maddox TW, Clegg PD, Diggle PJ, Wedley AL, Dawson S, Pinchbeck GL, Williams NJ. 2012. Cross-sectional study of antimicrobial-resistant bacteria in horses. Part 1: Prevalence of antimicrobial-resistant *Escherichia coli* and methicillin-resistant *Staphylococcus aureus*. Equine Vet J 44:289-296.

345 [24] Tenaillon 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.
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Figure legends:

Fig. 1. Analysis of *Xba*I-PFGE patterns obtained from ESBL/AmpC-producing *E. coli* isolates.

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

Fig. 2. Analysis of *Eco*RI-RFLP profiles of IncHI1 plasmids carrying $bla_{\text{CTX-M1/2}}$ genes. Coloured tree branches correspond to plasmids from the same animal (blue and green) or distinct animals from the same facility as well as from different facilities (red).* IncHI1 plasmids carrying $bla_{\text{CTX-M-2}}$. *fos* operon presence/absence, ■/□. IncHI1-ST9 plasmid pRCS78 was previously described and added as control (complete sequence available, GenBank accession number LT985296) (4). DNA of IncHI1 plasmids from *E. coli* isolates ABE1-40-R, AQE4-7-B, and NC0-17-R could not be restricted by *Eco*RI in repeated attempts, thus was excluded from the present analysis.

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

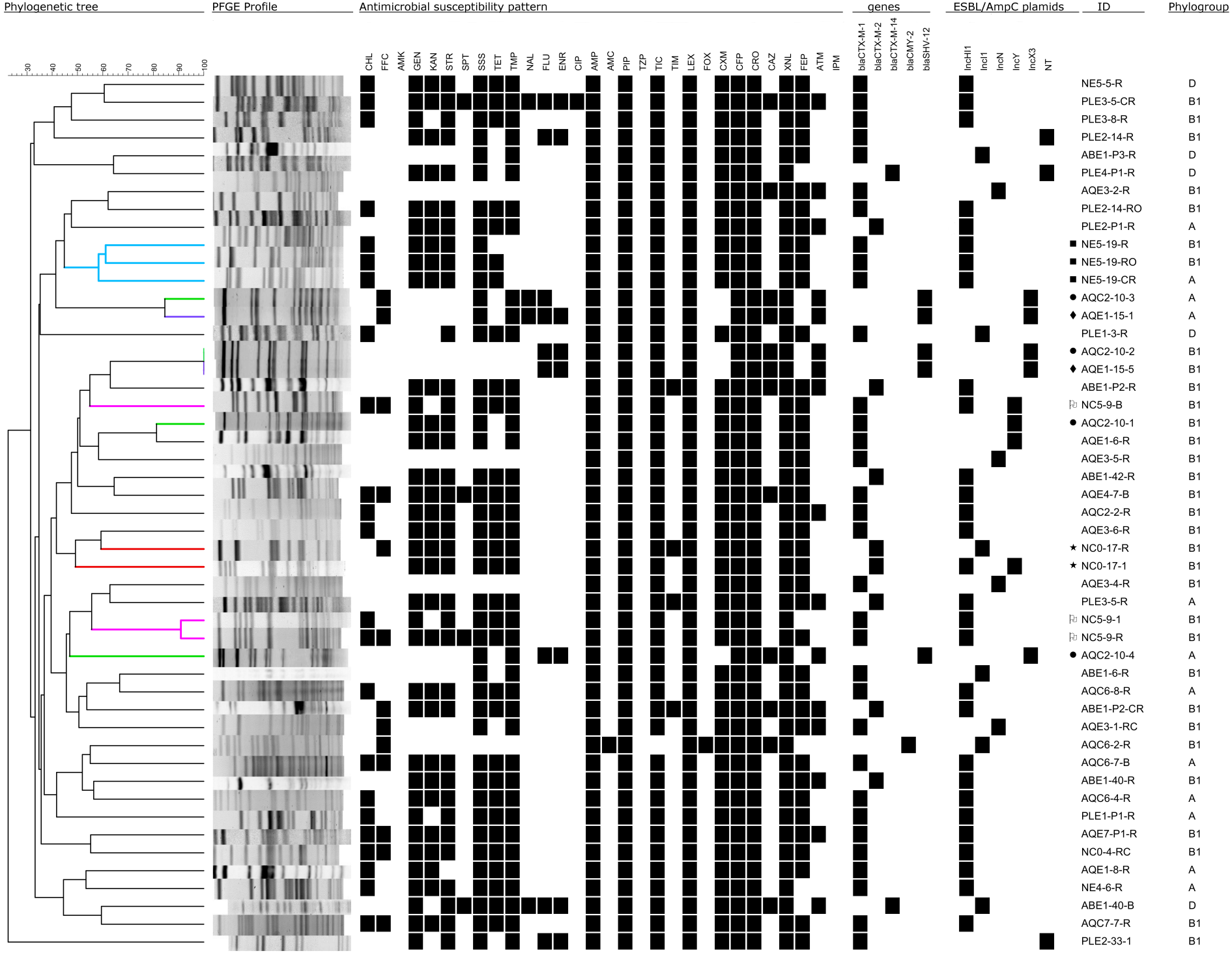
AQC6-7-B	CHL GEN KAN STR SUL TMP TET	*bla*$_{\text{CTX-M-1}}$	HI1-pST9*	CHL GEN KAN STR SUL TMP TET	+	+
AQC6-8-R	CHL GEN KAN STR SUL TMP TET	*bla*$_{\text{CTX-M-1}}$	HI1-pST9	CHL GEN KAN STR SUL TMP TET	+	+
AQC7-7-R	CHL GEN KAN STR SUL TMP TET	*bla*$_{\text{CTX-M-1}}$	HI1	CHL GEN KAN STR SUL TMP TET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

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

[a]Isolate 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 administrative regions (AB, Auvergne-Burgundy; AQ, Aquitaine; N, Normandy; PL, Pays-de-Loire). “En” or “Cn” correspond to the breeding facility (E) or

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 are several isolates from the same horse.

[b]NT, non-typeable. Asterisks indicate non-self-conjugative plasmids.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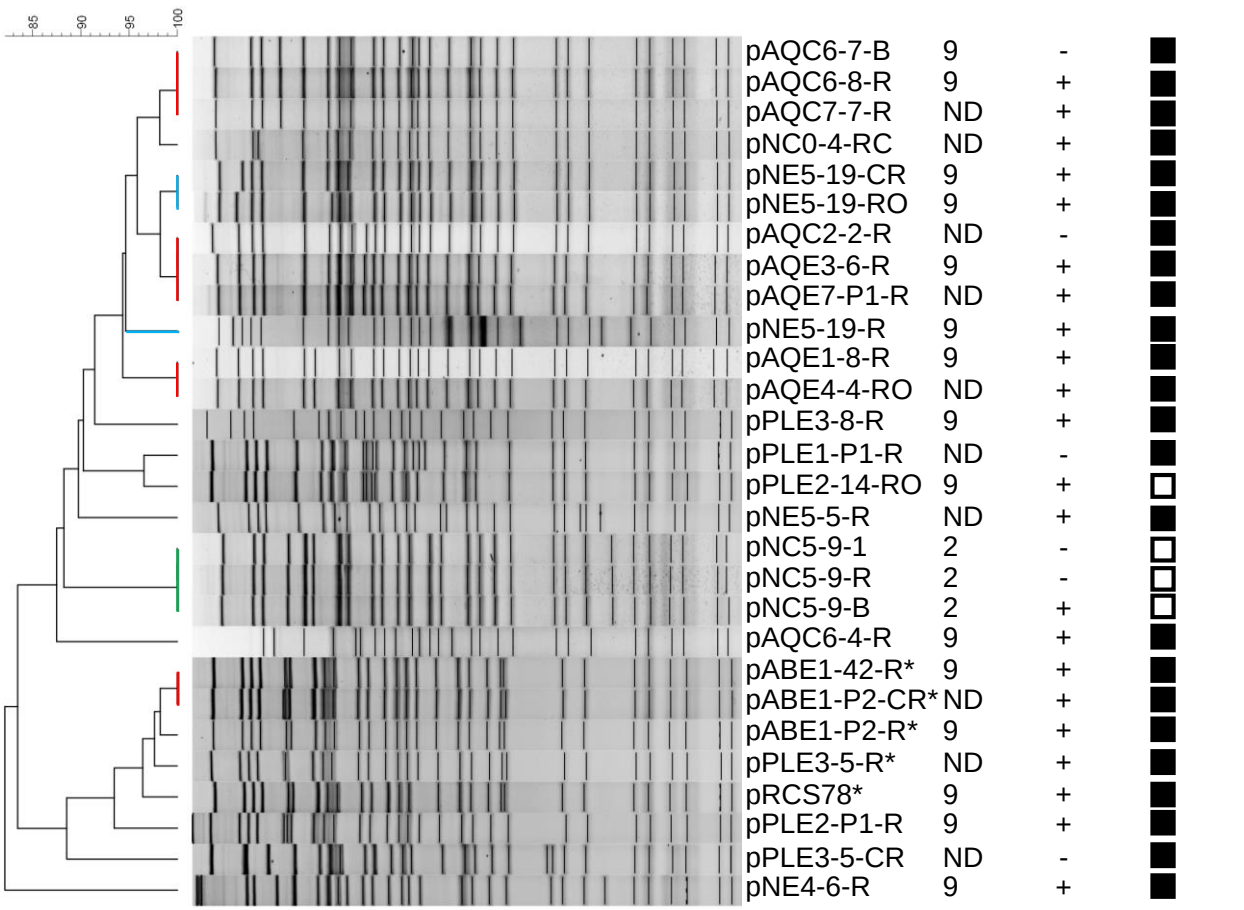
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pAQC7-7-R ND +
pNC0-4-RC ND +
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