Original research

Clones of enterotoxigenic and Shiga toxin-producing *Escherichia coli* implicated in swine enteric colibacillosis in Spain and rates of antibiotic resistance

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ABSTRACT

Shiga toxin–producing *E. coli* (STEC) and enterotoxigenic *E. coli* (ETEC) are the main agents of swine colibacillosis, an infectious disease which implies important economic losses. We characterized here 186 diarrheagenic *E. coli* from Spanish industrial pig farms (2005-2017) to know which clones were involved in this syndrome, and the rates of antibiotic resistance. The PCR based on pathotype-associated virulence genes determined that 161 of 186 isolates (86.5%) exhibited the ETEC pathotype, 10 (5.4%) the STEC pathotype, and 15 (8.1%) the hybrid ETEC/STEC pathotype. The majority of the isolates showed phylogroup A (85.5%), clonotype CH11-24 (72%) and belonged to the clonal complex (CC) 10, including two ETEC clones accounting for around 50% of the 186 isolates: O157:HNM-A-ST10 (CH11-24), which exhibited mostly the fimbrial antigen F4ac; and O108:HNM-A-ST10 (CH11-24), which exhibited mainly F18. Other associations were O139:H1-E-ST1 (CH2-54) with the STEC pathotype, and both O141:H4-A-CC10 (CH11-24) and O138:HNM-E-ST42 (CH28-41) with ETEC/STEC. We found that 87.1% of the isolates were multidrug-resistant, including 9% ESBL-producers, with the highest rates to nalidixic acid (82%), colistin (77%), ticarcillin (76%) and ampicillin (76%). Besides, more than 50% of isolates showed non-susceptibility to gentamicin, tobramycin, doxycycline, ciprofloxacin, trimethoprim-sufamethoxazole and chloramphenicol. Additionally, 11 out of 17 ESBL-producing isolates were *mcr*-carriers. Results suggest that O108:HNM-A-ST10 (CH11-24) F18 is an emerging clone taking space left by other classical serogroups. Further follow-up studies on predominant clones in pig colibacillosis are essential for the update of vaccines, as alternative to the use of antibiotics.

Keywords:

E. coli, swine colibacillosis, ETEC, STEC, F18, F4 (K88), antibiotic resistance.

1. Introduction

Swine colibacillosis is a multifactorial syndrome caused by *Escherichia coli*, which exhibits three main disease conditions, namely, edema disease, neonatal and post-weaning diarrhea. Each of them can be differenciated by the pathogenesis, age-range of the affected animals, and the involved pathotype: Shiga toxin–producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), and atypical enteropathogenic *E. coli* (aEPEC). Besides, hybrid strains (ETEC/STEC) are also relatively common (Fairbrother & Gyles, 2012; Gyles & Fairbrother, 2010; Mainil & Fairbrother, 2014). However, among all pathotypes, ETEC is the most prevalent cause of diarrhea in piglets (Dubreuil et al., 2016; García-Meniño et al., 2018).

In addition to enterotoxins (LT, STa and/or STb), the ETEC isolates exhibit colonization fimbriae which adhere to specific receptors of the small intestinal enterocytes of piglets. These fimbriae are F4 (K88), subtypes F4ab, F4ac and F4ad; F5 (K99); F6 (P987); F41 and F18, with three subtypes F18ab (F107), F18ac (2134P, 8813) and F18 new variant (Byun et al., 2013). Neonatal diarrhea affects suckling piglets during the first week of life, when there is a major development of the enterocyte receptors to F4, F5, F6, or F41 fimbriae (Fairbrother and Gyles, 2012; Mainil and Fairbrother, 2014). During the weaning period, different situations such as forced diet changes, trigger the apparition of post-weaning diarrhea (PWD) (Dubreuil et al., 2016). It is in this period when the piglets show a greater predisposition in the binding of F18 and/or F4 fimbriae to their receptors in the enterocyte (Curcio et al., 2017). Finally, edema disease is a toxemia caused by Stx2e-producing *E. coli* (STEC). The adhesion of Stx2e isolates to the enterocyte is mainly mediated by the F18 fimbriae (Fairbrother & Gyles, 2012; Gyles & Fairbrother, 2010).

Swine colibacillosis is one of the major challenges for the pig industry due to the high morbidity and mortality rates, as well as the cost derived from its prevention and antibiotic treatment. On the other hand, WHO currently identifies the spread of multiresistance, together with the decrease in the available antimicrobial treatments, as a main threat to the global health. WHO, together with ECDC and CDC, urge to implement a "One-Health" approach, involving human and veterinary medicine (WHO, 2019). The pig sector stands out as one of the largest consumers of antibiotics in food-production animals (EMA, 2019). In fact, there is an increasing trend in the detection of multidrug-resistant (MDR) ETEC strains of porcine origin (Aarestrup et al., 2008; Luppi, 2017; García-Meniño et al., 2018). The intensive use of antimicrobials in livestock implies a selection pressure that can boost the distribution, regrouping, and co-location of virulence and resistance genes in conjugative plasmids or pathogenicity islands (Shepard et al., 2012). Currently, vaccination is the most effective and economical method for the control of pig colibacillosis and the most appropriate alternative to reduce the excessive consumption of antibiotics in animal production (Melkebeek et al., 2013). So far, there are few studies focused on the identification and clone characterization of ETEC and STEC isolates involved in swine colibacillosis (Shepard et al., 2012; Abraham et al., 2014; Kusumoto et al., 2016). The aim of the present study was to contribute to this knowledge with the identification of the clonal groups involved in porcine colibacillosis in Spain, as well as their rates of antibiotic resistance.

2. Material and Methods

2.1 *E. coli* collection

One hundred and eighty-six *E. coli* isolates implicated in swine enteric colibacillosis, and collected during the years 2005 to 2017 in at least 18 Spanish provinces and 50 different Spanish farms, constituted the collection of study. The isolates were recovered from faecal swabs of pigs affected with diarrhea which were tested for routine diagnosis of enteric colibacillosis. Only one sample per animal was included in the study (Supplementary Table S1). Faecal swabs were plated on lactose MacConkey agar (LMAC) and sorbitol MacConkey agar (Oxoid) supplemented with cefixime (0.05 mg/l) and potassium tellurite (2.5 mg/l) (CTSMAC), and incubated at 37 °C for 18–24 h. Then, the confluent growth of all plates was tested to detect the presence of ETEC and STEC by PCR based on specific genes encoding toxins (LT, STa, STb and Stx2e) and fimbriae (F4, F5, F6, F18, and F41) (Supplementary Table S2). From positive confluents, a selection of up to five *E. coli*-like colonies plated on tryptone soy agar (Oxoid) were individually analyzed by PCR. Those colonies showing different genetic characteristics for the selected targets were stored at room temperature in nutrient broth (DifcoTM) with 0.75% nutrient agar (DifcoTM) for further characterization. The 186 *E. coli* represented at least one isolate per outbreak, with a maximum of three per outbreak and showing different serogroups and/or virulence profiles. From those 186, a group of 131 isolates had been analyzed in previous studies of colistin resistance associated to *mcr* gene (102 *mcr*-4 and 29 *mcr*-1 positive isolates) (García et al., 2018; García-Meniño et al., 2018).

2.2 O and H Typing

Determination of O:H antigens was carried out following the method described by Guinée et al., (1981) with O1 to O185 and H1 to H56 antisera, respectively. The antisera were obtained at the Laboratorio de Referencia de *E. coli* (LREC-USC). All antisera were absorbed with the corresponding cross-reacting antigens to remove the

nonspecific agglutinins. Isolates that did not react with any O antisera were classified as non-typeable (ONT), and non-motile strains (HNM) were further tested by PCR to determine their flagellar genes (Supplementary Table S2).

2.3 Antimicrobial Susceptibility and Genotypic Characterization of β-Lactamases

Antimicrobial susceptibility was determined by minimal inhibitory concentrations (MICs) using the MicroScan WalkAway®-automated system (Siemens Healthcare Diagnostics, CA, United States) according to the manufacturer's instructions. The antibiotics tested included: aztreonam, cefepime, ceftazidime, ticarcillin, ampicillin-sulbactam, piperacillin-tazobactam, imipenem, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, trimethoprim-sulphamethoxazole, fosfomycin, colistin, minocycline and tigecycline. Additionally, resistance to amoxicillin+clavulanic acid, ampicillin, cefazolin, cefotaxime, cefoxitin, cefuroxime, chloramphenicol, doxycycline, nalidixic acid and nitrofurantoin was determined by disk (Becton Dickinson, Sparks, MD, United States) diffusion assays. All results were interpreted according to human specific beakpoints of the CLSI (2020) except for ticarcillin and tigecycline values, which were interpreted according to EUCAST (2020). MDR isolates were defined following the Magiorakos et al. (2012) criteria, as those showing acquired non-susceptibility to at least one agent in three or more antimicrobial categories. Besides, isolates fenotipically suspected of ESBL production were tested for ESBL-encoding genes: *bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$ and *bla*$_{\text{CTX-M}}$ followed by *bla*$_{\text{CTX-M}}$ group 1 and group 9 by PCR amplification and sequencing of the positive amplicons using specific primers and conditions described elsewhere (Supplementary Table S2).

2.4 Phylogroups, Clonotypes, and Sequence Types

The main phylogenetic groups of *E. coli* (A, B1, B2, C, D, E, and F) were assigned by means of the quadruplex PCR method described by Clermont et al., (2013)

(Supplementary Table S3). Then, the sequence type (ST) of a selection of 88 isolates was determined by Multilocus Sequence Typing (MLST) following the Achtman seven-locus scheme (Wirth et al., 2006). Primers, conditions and the allelic profile were retrieved through the Enterobase website (https://enterobase.warwick.ac.uk/species/ecoli/allele_st_search) (Supplementary Table S3). The 88 isolates included representatives of the different phylogroup/serogroup/clonotype combinations found within the 186 *E. coli*. To confirm the consistency of the phylogroup assignations, a neighbor-joining tree was constructed by MEGA6 (Tamura et al., 2013), based on concatenated sequences of the seven housekeeping genes from the MLST scheme. Finally, the clonotyping was based on the internal 469-nucleotide (nt) and 489-nt sequence of the *fumC* and *fimH* genes, respectively (Weissman et al., 2012), where the combination of *fumC* (from MLST) and *fimH* (alleles obtained from FimTyper database https://cge.cbs.dtu.dk/services/FimTyper/) determined the clonotype (CH) (Supplementary Table S3).

2.5 Statistical Analysis

The differences within groups were compared by a one-tailed Fisher's exact test. *P* values <0.05 were considered statistically significant.

3. Results

3.1 Pathotypes and virulence profiles

PCR based on specific genes encoding the LT, STa, STb and Stx2e toxins determined that 161 of 186 isolates (86.5%) conformed to ETEC pathotype (positive for genes encoding enterotoxins, *eltA* and/or *estA*, and/or *estB*). Besides, 10 isolates (5.4%)

conformed to STEC pathotype (positive for gene encoding the Shiga toxin *stx2e*) and 15 isolates (8.1%) were of the hybrid ETEC/STEC pathotype (positive for both Shiga toxin and enterotoxin encoding genes) (Table 1). The STb-encoding gene was the most frequently detected within the collection (74.7%), followed by LT (66.1%) and STa (50.5%), and only 13.5% of the strains were carriers of the Stx2e-encoding gene. With regard to the intestinal colonization factors, F18 was the most prevalent fimbriae-encoding gene (43.5% of the 186 isolates), followed by F4 (38.2%) of the F4ac subtype. Finally, four isolates encoded the F5 fimbriae, two the F6, and five carried both the F5- and F41-encoding genes. The most common virulence profiles within each pathotype were: LT, STb, F4 and LT, STa, STb, F18 (37.3% and 18.6% of the 161 ETEC isolates, respectively); STa, STb, Stx2e, F18 (86.7% of the 15 ETEC/STEC isolates) and Stx2e, F18 (70% of the 10 STEC isolates) (Table 1).

3.2 Serotyping

Twenty-three different O:H combinations (serotypes) were determined (Supplementary Table S1). However, four of 23 accounted for 68.3% of the 186 isolates: O157:HNM (24.7%; 46 isolates), O108:HNM, (23.1%; 43 isolates), O141:H4 (10.8%; 20 isolates) and ONT:HNM (9.7%; 18 isolates). A high number of isolates were non-motile (HNM) and negative for the *fliC*$_{H}$ genes analyzed by PCR (68.3%; 127 strains). Importantly, we found significant associations between certain serotypes and pathotypes: O157:HNM and O108:HNM with ETEC, O141:H4 and O138:HNM with ETEC/STEC and O139:H1 with STEC (P <0.05 for all comparisons) (Table 2). Furthermore, there was a significant correlation between the carriage of F4-encoding gene and specific serotypes: O157:HNM, O149:H10 and O45:HNM (84.8%, 100% and 85.7% of isolates belonging to those serotypes were F4 positive, respectively). Likewise, the F18 showed correlation with serotypes O141:H4, O139:H1, O138:HNM and O108:HNM (75%, and 87.5%,

90% and 79.1% of isolates belonging to those serotypes were F18 positive, respectively) (P <0.05 for all comparisons). Finally, seven of the nine F5 and/or F41 isolates of this study belonged to the serogroup O101 with different H antigen combinations (H10, H9 or HNM) (Table 2).

3.3 Clones involved in pig colibacillosis

By the quadruplex PCR method described by Clermont et al., (2013), three pylogroups were identified within the 186 isolates, although the majority belonged to phylogroup A (85.5%; 159 isolates), followed by E (12.4%; 23 isolates) and B1 (2.1%; 4 isolates). The ETEC isolates clearly belonged to phylogroup A (93.2%; 150 of 161), while the STEC pathotype appears associated to phylogroup E (90%; 9 of 10) (P values <0.05). Clonotyping identified 14 different clonotypes, however, CH11-24 was by far the most prevalent (72%; 134 isolates), followed by CH27-0 (5.9%; 11 isolates), CH11-54 (4.8%; 9 isolates), CH28-41 (4.8%; 9 isolates) and CH2-54 (4.8%; 9 isolates). Most ETEC showed CH11-24 (77.6%), while CH11-24 and CH28-41 were similarly represented within ETEC/STEC (53.3% and 46.7%, respectively); and CH2-54 was the main clonotype established for STEC (90%) (Table 2; Supplementary Table S1).

By MLST, we further analyzed a selection of 88 isolates for their STs. This selection included representatives of the different phylogroup/serogroup/clonotype combinations found within the 186 *E. coli*. As a result, we obtained 16 alellic profiles, 10 of which belonged to the CC10, accounting for 70.5% of the isolates (62 out of 88). Furthermore, the ST10 represented more than 50% (48 of 88). The remaining CC10 isolates showed: ST7323 (4), ST744 (2), ST5786 (2) and ST746, ST772, ST5507, STNew1, STNew2, STNew3 (1 isolate each). The other non-CC10 STs determined were: ST42 (11

224 isolates), ST1 (6), ST100 (6), ST58 (1), ST453 (1), ST910 (1) (Supplementary Table
225 S4).
226 The serotype, phylogroup, ST and clonotype typing allowed the definition of major
227 clones (Table 2). It is of note that two ETEC clones accounted for around 50% of the
228 186 isolates: O157:HNM-A-ST10 (CH11-24) and O108:HNM-A-ST10 (CH11-24).
229 Other significant associations were O139:H1-E-ST1 (CH2-54) with the STEC
230 pathotype, and both O138:HNM-E-ST42 (CH28-41) and O141:H4-A-CC10 (CH11-24)
231 with ETEC/STEC ($P < 0.05$). The association found between fimbriae and clones was
232 also of great interest: F18 with clones O108:HNM-A-ST10 (CH11-24), O141:H4-A-
233 CC10 (CH11-24), O138:HNM-E-ST42 (CH28-41) and O139:H1-E-ST1 (CH2-54); and
234 F4ac with O157:HNM-A-ST10 (CH11-24), O149:H10-A-ST100 (CH27-0) and
235 O45:HNM-A-ST10 (CH11-24) ($P < 0.05$). The F5 and/or F41-encoding genes were
236 detected in clones O101:H9-A-ST10 (CH11-54), O101:H9-A-ST10 (CH11-399),
237 O101:H10-A-ST744 (CH11-54), O101:HNM-A-ST10 (CH11-54) (Table 2).
238
239 **3.4 Antibiotic resistance and ESBL-typing**
240 Of the 186 *E. coli* isolates, 162 (87.1%) showed acquired non-susceptibility to at least
241 one agent in three or more antimicrobial categories, and therefore were defined as MDR
242 (Magiorakos et al., 2012). Furthermore, 55.9% of the isolates were non susceptible to ≥
243 six antimicrobial categories (Table 3). The highest rates of resistance were to nalidixic
244 acid (82.3%; 153 isolates), colistin (76.9%; 143 isolates), ticarcillin (76.3%; 142
245 isolates) and ampicillin (76.3%; 142 isolates). Furthermore, more than 50% of isolates
246 were non-susceptible to ampicillin/clavulanic acid, gentamicin, tobramycin,
247 doxycycline, ciprofloxacin, trimethoprim-sufamethoxazole and chloramphenicol.

248 Globally, ETEC isolates showed a significant higher rate of resistance in comparison
249 with STEC (Table 3).
250 Besides, 17 (9.1%) of the 186 *E. coli* were ESBL-producers. The ESBL-typing showed
251 the *bla*$_{\text{CTX-M-14}}$ gene in eight isolates, *bla*$_{\text{SHV-12}}$ in six, *bla*$_{\text{CTX-M-1}}$ in three, and *bla*$_{\text{TEM-135}}$
252 in one of the *bla*$_{\text{CTX-M-14}}$-positive isolates. Eight of the 17 ESBL-producers belonged to
253 clones O108:HNM-A-ST10 (CH11-24) (5 isolates) and O157:HNM-A-ST10 (CH11-
254 24) (3 isolates). Furthermore, 11 of the 17 ESBL-producers were *mcr*-carriers (Table 4).
255

256 **4. Discussion**

257 Enteric colibacillosis in pig industry is still highly prevalent and associated to important
258 economic looses worldwide. Vaccination is, in addition to antimicrobial treatment, one
259 important part in controlling disease. But the continuing abuse of the latter has been
260 highlighted as a main cause of the AMR increase, including resistance to colistin
261 (Curcio et al., 2017; Luppi, 2017). The detailed characterization of *E. coli* clones
262 involved in swine enteric colibacillosis is critical for the effective surveillance and
263 design of alternatives to the use of antibiotics. So, the aim of the present study was the
264 clone characterization of the classical pathotypes ETEC and STEC involved in swine
265 colibacillosis. The aEPEC-associated genes and their clones were not investigated here.
266 While it is true that aEPEC have also been implicated in PWD in pigs (García-Meniño
267 et al., 2018), their pathogenicity still remains unclear (Malik et al., 2017).
268 We defined the clonal structure of the pathogenic *E. coli* collection by means of the
269 serotype-phylogroup-ST-CC-CH and virulence typing combination. Serogroups such as
270 O8, O138, O139, O141, O147, O149 and O157, have been traditionally reported in
271 swine colibacillosis worldwide (Garabal et al., 1996; Gyles and Fairbrother, 2010;
272 Mainil and Fairbrother, 2014). In Spain, the serogroups O9, O20, O101, O138, O141

and O149, identified within the isolates of this study, were also present in the 80s and 90s, although showing different prevalence. The O9, O20, O101, O138, O141 and O149 were associated with ETEC, and the O91 and O138 with STEC isolates (Garabal et al., 1996). In a subsequent paper, the characterization of 74 representative isolates showed the presence of three major seropathotypes: O141:HNM:K85ab, F6 (12.2%; 9/74); O149:H10:K91, LT, F4 (12.2%; 9/74) and O138:H14:K81, STx (6.8%; 5/74) (Blanco et al., 1997). In addition to those, we report here the emergence of serogroups O157 and O108. In fact, O157:HNM (24.7%), O108:HNM (23.1%), O141:H4 (10.8%) and ONT:HNM (9.7%) were the four major serotypes accounting for 68.3% of the 186 isolates characterized in the present study. Therefore, we have detected in Spain an evolution in the prevalence of the serogroups traditionally reported in swine colibacillosis, mainly due to the emergence of O108. There are few reports of this serogroup within swine *E. coli* isolates. Salajka et al., (1992) detected the O108 in 11.3% of 943 strains (fifth in importance) from pigs with edema disease in Czech Republic. Brand et al., (2017) identified an O108 ETEC isolate (STa, EAST-1) in a study carried out in Switzerland. Besides, Magistrali et al., (2018) reported the presence of two O108:H39-ST10 *mcr*-positive isolates in pigs with colibacillosis (one from Spain and one from Belgium). Studies on pig STEC show a more limited range of serogroups (O138, O139 and O141) (Mattsson and Wallgren, 2008; Gyles and Fairbrother, 2010). In agreement, all our STEC isolates belonged to those three serogroups. In any case, there is a global lack of information regarding serotypes/O:H antigens of *E. coli* currently implicated in swine colibacillosis.

Despite the genetic diversity showed by the 186 isolates of our collection, belonging to three phylogroups and 13 clonotypes, we observed a clonal structure within the diarrheagenic pathotypes. Thus, most ETEC isolates belonged to the CC10-A (CH11-

24) clonal group, the majority of STEC isolates exhibited ST1-E (CH2-54), and the hybrid STEC/ETEC isolates appeared distributed within CC10-A (CH11-24) and ST42-E (CH28-41). By far, the clonal group CC10-A (CH11-24) was the most prevalent, accounting for 72% of the isolates. The CC10 is found widely disseminated and very successful in the intestinal microbiota of humans, food-production animals and the environment (river water and wastewater) (Varela et al., 2015). However, CC10 contains a high phylogenetic diversity in number of STs (Reid et al., 2018). Within CC10, and in accordance with the results presented here, is ST10 one of the most commonly STs reported in swine colibacillosis (Shepard et al., 2012; Yang et al., 2019). Other authors have also found certain clonality within swine pathogenic *E. coli* isolates in different countries. Thus, Shepard et al., (2012) conducted a phylogenetic analysis of 78 strains from suckling and post-weaned piglets, and identified three major clonal groups (ST10-CC10, ST23-CC23 and ST169) associated with swine colibacillosis in USA. In piglets with diarrhea or edema disease in Japan, O139-ST1 (26.1%) and O149-ST100-CC165 (25.3%) isolates were the most frequently detected (Kusumoto et al., 2016). A more recent study in China, highlighted the importance of ST10 (CC10) (12.8%) and ST48 (CC10) isolates (9.4%) (Yang et al. 2019). In Australia, Abraham et al., (2014) identified O149-A-ST100-CC165 (44.3%) and O141-A-ST1260 (CC10) (38,6%) as the most prevalent clones among 70 ETEC isolates (seven of neonatal diarrhea and 63 of post-weaning diarrhea) recovered between 1999 and 2005. The O149:H10-A-ST100-CC165 (CH27-0) F4ac clone was also identified in 54.4% of 90 PWD isolates of a recent study performed in Denmark (García et al., 2020). The A-ST100 (CH27-0) F4ac comprised, however, only 4.3% of our collection (11 out of 186 isolates) including clones: O149:H10-A-ST100 (8 isolates) and O138:H10-A-ST100 (3 isolates). The Spanish picture regarding antibiotic resistance is also very different to that found in Denmark, where no resistance

was found to ciprofloxacin or colistin (García et al., 2020). On the contrary, the prevalence within our collection to these antibiotics were 56% and 77%, respectively. The widespread use of antibiotics in animal and human medicine has contributed to the high rates of antibiotic resistance worldwide. Vieira et al., (2011) reported a significant association in many European countries between antimicrobial resistance in *E. coli* isolates from food animals and clinical human samples, especially regarding resistance to quinolones, fluoroquinolones, beta-lactams and aminoglycosides. The European Medicines Agency (EMA) has recently proposed a new categorization of antimicrobials (EMA, 2020). This proposal establishes four categories, with Category A ("Avoid") including those antimicrobials not currently authorized in veterinary medicine in the European Union, such as fosfomycin or monobactams; and with Category B ("Restrict") including those that should be restricted in animals to mitigate the risk to public health, namely, quinolones, 3rd- and 4th-generation cephalosporins and polymyxins. The high resistance rates detected here to some of these antibiotics, with more than 50% of isolates non-susceptible to fluoroquinolones or colistin and 87% MDR, are therefore worrisome.

5. Conclusion

We have detected a shift in the predominant clones implicated in swine colibacillosis in Spain (2005-2017) compared to previous studies (1986-1991). Currently, there are two clones involved in around 50% of porcine colibacillosis in Spain: O157:HNM-A-ST10 (CH11-24) and O108:HNM-A-ST10 (CH11-24) associated with the fimbrial antigens F4ac and F18, respectively. Follow-up studies of predominant clones within pig colibacillosis are essential for the development of new vaccines, as alternative to the use of antibiotics.

6. Declarations:

None declared.

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Competing interests:

None declared.

Ethical approval:

Not required.

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