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Enterotoxigenic *Escherichia coli* Subclinical Infection in Pigs: Bacteriological and Genotypic Characterization and Antimicrobial Resistance Profiles

Fabiana A. Moredo,[1,*] Pablo E. Piñeyro,[2,*] Gabriela C. Márquez,[3,**] Marcelo Sanz,[4] Rocío Colello,[4] Analía Etcheverría,[4] Nora L. Padola,[4] María A. Quiroga,[5] Carlos J. Perfumo,[5] Lucía Galli,[6] and Gerardo A. Leotta[6]

Abstract

Enterotoxigenic *Escherichia coli* (ETEC) is the major pathogen responsible for neonatal diarrhea, postweaning diarrhea, and edema disease in pigs. Although it can be harmless, ETEC is also present in the intestines of other animal species and humans, causing occasional diarrhea outbreaks. The evaluation of this pathogen's presence in food sources is becoming an increasingly important issue in human health. In order to determine the prevalence of ETEC in nondiarrheic pigs, 990 animals from 11 pig farms were sampled. Using end-time polymerase chain reaction (PCR), *eltA*, *estI* genes, or both, were detected in 150 (15.2%) animals. From the positive samples, 40 (26.6%) ETEC strains were isolated, showing 19 antibiotic-resistance patterns; 52.5% of these strains had multiple antibiotic resistances, and 17.5% carried the *intI2* gene. The most prevalent genotypes were rfb_{O157}*/estII/aidA* (32.5%) and *estI/estII* (25.0%). The *estII* gene was identified most frequently (97.5%), followed by *estI* (37.5%), *astA* (20.0%), and *eltA* (12.5%). The genes coding the fimbriae F5, F6, and F18 were detected in three single isolates. The *aidA* gene was detected in 20 ETEC strains associated with the *estII* gene. Among the isolated ETEC strains, stx_{2e}*/estI*, stx_{2e}*/estI/estII*, and stx_{2e}*/estI/estII/intI2* genotypes were identified. The ETEC belonged to 12 different serogroups; 37.5% of them belonged to serotype O157:H19. Isolates were grouped by enterobacterial repetitive intergenic consensus–PCR into 5 clusters with 100.0% similarity. In this study, we demonstrated that numerous ETEC genotypes cohabit and circulate in swine populations without clinical manifestation of neonatal diarrhea, postweaning diarrhea, or edema disease in different production stages. The information generated is important not only for diagnostic and epidemiological purposes, but also for understanding the dynamics and ecology of ETEC in pigs in different production stages that can be potentially transmitted to humans from food animals.

Introduction

ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC) is the major pathogen responsible for neonatal diarrhea (ND), postweaning diarrhea (PWD), and edema disease (ED) in pigs (Fairbrother *et al.,* 2005; Nagy and Fekete, 2005; Fairbrother and Gyles, 2012). ETEC is also present in the intestines of other animal species and humans. Although it can be harmless, ETEC is considered one of the most common pathogens, causing diarrhea among travelers and children in developing countries. Infection is normally associated with the presence of ETEC in contaminated food or water (Qadri *et al.,* 2005; Zhang *et al.,* 2008). The evaluation of this pathogen's presence in food sources is becoming an increasingly important issue in human health.

ETEC-induced diarrhea holds negative economic implications for the pig industry due to the associated high mortality and reduced growth rate (Zhang *et al.,* 2007). ETEC

[1]Cátedra de Microbiología and [5]Instituto de Patología Dr. B. Epstein, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Buenos Aires, Argentina.
[2]Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa.
[3]Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, Virginia.
[4]Laboratorio de Inmunoquímica y Biotecnología, CIVETAN-CONICET/CIC/FCV-UNCPBA, Tandil, Buenos Aires, Argentina.
[6]Instituto de Genética Veterinaria ''Ing Fernando Noel Dulout,'' CCT-La Plata, CONICET, FCV-UNLP, Buenos Aires, Argentina.
*These two authors contributed equally to this work.
**Current address: Genus, PLC, DeForest, Wisconsin.

strains adhere to the small intestinal microvilli via fimbriae, such as F4, F5, F6, F18, and F41, encoded by *faeG, fanC, fasA, fedA*, and *F41* genes, and produce enterotoxins that act locally on enterocytes. Based on their thermal stability, ETEC enterotoxins are classified as heat-labile toxins (LT-I and LT-II), encoded by *eltA* and *eltB* genes or heat-stable toxins (STa, STb and EAST1) encoded by *estI, estII,* and *astA* genes (Dubreuil, 2008). These toxins are the primary virulence traits responsible for diarrhea; however, the presence of virulence factors such as the EAST1 toxin (Vu Khac *et al.,* 2006), the adhesin involved in diffuse adherence (AIDA-I), and the porcine attaching and effacing-associated factor (Paa) encoded by *aidA* and *paa* genes, have been implicated in porcine diarrhea (Ngeleka *et al.,* 2003; Sherlock *et al.,* 2004; Zhang *et al.,* 2007). None of these potential virulence factors have been well characterized for their significance in porcine diarrhea, and studies of their association with other virulence factors are limited (Ngeleka *et al.,* 2003; Chapman *et al.,* 2006; Zhang *et al.,* 2007).

Antimicrobial agents are widely used in swine production as therapeutic agents and growth promoters, and many of these antibiotics are important in human clinical medicine (Mathew *et al.,* 2007). Recent studies have shown that ETEC serotypes have developed resistance to antimicrobial agents used in human and veterinary medicine (Smith *et al.,* 2010; Wang *et al.,* 2011). The dissemination and acquisition of such genes by horizontal gene transfer has led to the rapid emergence of antibiotic resistance among bacteria (Carattoli, 2013). Class 1 and 2 integrons are the most prevalent in isolates and are largely implicated in the dissemination of antibiotic resistance (de la Torre *et al.,* 2014).

The purpose of this study was to investigate (1) the prevalence of ETEC-positive pigs in 11 farms without clinical signs of diarrhea, (2) the phenotypic and genotypic characterization of ETEC isolated from nondiarrheic pigs, and (3) antibiotic resistance among isolated ETEC strains.

Materials and Methods

Study design and sample collection

A cross-sectional study was carried out on 11 farms located in Buenos Aires, Argentina, with no history or clinical signs of colibacillosis. Swine units were included by 1 of the following criteria: sow herd size (225 ± 30) and finisher pigs delivered to slaughter/year (at least 2500 finisher pigs to market/year). The selected farms have the same management style and are farrow-to-finish. The geographic distribution represents the most swine-dense areas of Buenos Aires. Ninety rectal swabs were collected from each farm to determine the presence of *eltA, estI,* or both, and the subsequent prevalence of different genotypes in each production stage. Pigs considered being ETEC positive ($ETEC^+$) were those in which *eltA, estI,* or both were detected. The DNA template was prepared from the confluent growth zone. Thirty randomly selected pigs from each production stage were sampled as follows: farrowing (weaning piglet, 21 ± 3 days old); nursery (nursery piglets, 86 ± 3 days old); and finishing (finishing pigs, 165 ± 3 days old). Samples were collected over a period of 16 weeks. Samples consisted of rectal swabs (EUROTUBO® Collection Swab; DELTALAB, Barcelona, Spain), which were stored at 4°C and processed within 24 h.

Extraction of bacterial DNA and screening of eltA *and* estI *genes from nondiarrheic pigs*

A total of 990 rectal swabs were tested for the presence of ETEC genes by PCR detection of *eltA* and *estI*. Rectal swabs were streaked onto MacConkey Agar plates (Britania, Buenos Aires, Argentina) and incubated at 37°C for 18 h. Each sample was homogenized by inoculation of a loopful of the confluent growth zone in 3 mL of tryptic soy broth (TSB) (Britania). From each bacterial suspension, 700 μL was frozen at −70°C in 30.0% glycerol. DNA extraction was performed using 150 μL of bacterial suspension, as previously described (Leotta *et al.,* 2005). The PCR protocols have been previously described (Toma *et al.,* 2003). The strains *E. coli* ATCC 33965 (*eltA*/*estI*) and *E. coli* ATCC 25922 were used as positive and negative controls, respectively.

Positive ETEC isolation, biochemical characteristics, and hemolysin activity

Frozen stocks of samples positive for the *eltA, estI,* or both were inoculated in TSB and incubated at 42°C for 24 h. Isolates of positive samples were made by streaking the enrichment culture onto one MacConkey Agar plate (Britania) and three Eosin Methylene Blue (EMB) Agar plates (Britania). A total of 60 individual colonies per sample, with *E. coli* morphology, were tested for the presence of virulence genes. One positive isolate per sample for *eltA, estI,* or both was confirmed as *E. coli* by standard biochemical tests (Brusa *et al.,* 2013).

Conventional serotyping

The presence of O and H antigens was determined by a previously described method (Guinée *et al.,* 1981) with slight modifications (Blanco *et al.,* 1997) in which all available O (O1-O181) and H (H1-H56) antisera were used (Orskov *et al.,* 1984). Nonspecific agglutinins were removed by adsorption of each antiserum with the corresponding cross-reacting antigens. The O and H antisera were produced in the Laboratorio de Referencia de *E. coli* (LREC), Universidade de Santiago de Compostela (Lugo, Spain).

Antimicrobial susceptibility testing

Antimicrobial susceptibility was tested using the disk-diffusion method following the Clinical and Laboratory Standards Institute standard M31-A3 and M100-S23 (CLSI, 2008, 2013), for the following antimicrobial agents: ampicillin (AMP), cephalothin (CET), cefotaxime (CTX), cefoxitin (CXT), amoxicillin/clavulanic acid (AMC), gentamicin (GEN), amikacin (AMK), streptomycin (STR), tetracycline (TET), nalidixic acid (NAL), ciprofloxacin (CIP), chloramphenicol (CHL), florfenicol (FFN), trimethoprim-sulfamethoxazole (SXT), nitrofurantoin (NIT), fosfomycin (FOF), and colistin (CST) disk (Britania). The manufacture guidelines were used for CST interpretation. *E. coli* ATCC 25922 was used as reference strain. Multiple antibiotic resistance (MAR) was defined as ETEC isolates showing three or more antimicrobial classes resistance (Schwarz *et al.,* 2010).

Genotypic characterization

According to references included in Table 1, PCR testing was performed to detect the presence of genes coding for LT,

TABLE 1. VIRULENCE AND INTEGRONS GENES TARGETS AND PRIMERS SEQUENCES USED TO CHARACTERIZE ENTEROTOXIGENIC *ESCHERICHIA COLI* ISOLATED FROM NONDIARRHEIC PIGS

Virulence factors and integrons	*Gene*	*Oligonucleotide sequence (5′-3′)* *Sense*	*Anti-sense*	*Fragment size (bp)*	*Reference*
LTa	*eltA*	TTAATAGCACCCGGTACAAGCAGG	CCTGACTCTTCAAAGAGAAAATTAC	147	Toma *et al.*, 2003
STa	*estI*	TCTCTATGTGCATACGGAGC	CCATACTGATTGCCGCAAT	322	Toma *et al.*, 2003
O157	rfb_{O157}	CGGACATCCATGTGATATGG	TTGCCTATGTACAGCTAATCC	259	Leotta *et al.*, 2005
F4	*faeG*	GGTGATTTCAATGGTTCG	ATTGCTACGTTCAGCGGAGCG	764	Chapman *et al.*, 2006
F5	*fanC*	TGGGACTACCAATGCTTCTG	TATCCACCATTAGACGGAGC	450	Chapman *et al.*, 2006
F6	*fasA*	TCTGCTCTTAAAGCTACTGG	AACTCCACCGTTTGTATCAG	333	Chapman *et al.*, 2006
F18	*fedA*	GTGAAAAGACTAGTTTTATTTC	CTTGTAAGTAACCGCGTAAGC	510	Chapman *et al.*, 2006
F41	*F41*	GAGGGACTTTCATCTTTTAG	AGTCCATTCCATTTATAGGC	431	Chapman *et al.*, 2006
Paa	*paa*	ATGAGGAAACATAATGGCAGG	TCTGGTCAGGTCGTCAATAC	350	Chapman *et al.*, 2006
AidA	*aidA*	ACAGTATCATATGGAGCCA	TGTGCGCCAGAACTATTA	585	Ngeleka *et al.*, 2003
Intimin	*eae*	CCCGAATTCGGCACAAGCATAAGC	CCGGATCCGTCTCGCCAGTATTCG	864	Karch *et al.*, 1993
LTa	*eltA*	GGCGACAGATTATACCGTGC	CCGAATTCTGTTATATATGTC	696	Chapman *et al.*, 2006
STa	*estI*	TCTTTCCCCTCTTTTAGTCAG	ACAGGCAGGATTACAACAAAG	166	Chapman *et al.*, 2006
STb	*estII*	ATCGCATTTCTTCTTGCATC	GGGCGCCAAAGCATGCTCC	172	Chapman *et al.*, 2006
EAST1	*astA*	CCATCAACACAGTATATCCGA	GGTCGCGAGTGACGGCTTTGT	111	Chapman *et al.*, 2006
Stx2e	stx_{2e}	CCTTAACTAAAGGAATATA	CTGGTGGTGTATGATTAATA	230	Blanco *et al.*, 1997
IntI1	*intI1*	ATCATCGTCGTAGAGACGTCGG	GTC AAG GTT CTG GAC CAG TTG C	892	Rosser *et al.*, 1999
IntI2	*intI2*	GCAAATGAAGTGCAACGC	ACACGCTTGCTAACGATG	700	Orman *et al.*, 2002

STa, STb, Stx1, Stx2, Stx2e, and EAST1 toxins, as well as Class 1 and Class 2 integrons (*intI1* and *intI2*), and F4, F5, F6, F18, F41, intimin, Paa and AIDA-I adhesins, and the rfb_{O157} gene. Reference strains *E. coli* EDL933 (*eae*/*paa*/rfb_{O157}), *E. coli* 7805 (*eltA*/*estI*/*estII*/*faeG*/*east1*), *E. coli* 81-603 A (*fasA*), *E. coli* 1073 B44 (*fanC*/*F41*), and *E. coli* 88-1199 (*fedA*) were used as positive controls. ETEC isolates were further characterized by enterobacterial repetitive intergenic consensus (ERIC)–PCR (Versalovic *et al.,* 1991). ERIC fingerprints of amplified DNA fragments were obtained by agarose gel electrophoresis and analyzed using the software BioNumerics Version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium).

Statistical analysis

Within-herd prevalence for each genotype in different production stages was calculated by dividing the number of ETEC-positive samples by each genotype identified. Ninety-five percent confidence intervals were computed for all of these estimates.

Results

Prevalence of eltA *and* estI *genes in nondiarrheic pigs*

Of the 990 samples screened, 150 (15.2%; 95.0% CI ± 2.24) samples were positive for the presence of *eltA*, *estI*, or both. Of these $ETEC^+$ pigs, 82.7% (95.0% CI ± 6.05) were positive for *estI*, 11.3% (95.0% CI ± 5.07) were positive for *eltA*, and 6.0% (95.0% CI ± 3.8) were positive for *estI*/*eltA* (Fig. 1). The overall percentage of $ETEC^+$ animals increased from 16.6% (95.0% CI ± 5.95) in the farrowing phase to 66.0% (95.0% CI ± 7.58) in the nursery phase. The percentage of carrier pigs abruptly declined to 17.3% (95.0% CI ± 6.05) in the finisher population. $ETEC^+$ pigs were present in 10 of the 11 evaluated farms. Among the 150 $ETEC^+$ pigs, 40 ETEC strains (26.7%) (95.0% CI ± 13.71) were isolated. ETEC strains were isolated from 30 nursery samples (75.0%) (95.0% CI ± 13.42), 8 finishing samples (20.0%) (95.0% CI ± 12.4), and 2 farrowing samples (5.0%) (95.0% CI ± 6.75).

Serotype profiles of the ETEC strains

The ETEC strains were grouped in 12 different serotypes, with O157:H19 being the most prevalent ($n = 15$; 37.5%), followed by serotypes O8:H- ($n = 7$; 17.5%), ONT:H- ($n = 5$; 12.5%), O8:H31 ($n = 4$; 10.0%), O8:H26 ($n = 2$; 5.0%), and individual cases of serotypes O7:H-, O7:H15, O7:H16, O54:H21, O60:H-, O149:H-, and O157:H-. Of the nontypeable somatic strains, all were nonmotile (Fig. 2).

Antimicrobial susceptibility of ETEC strains

Two of the 40 ETEC strains were susceptible to all antimicrobial agents tested. All of the strains were susceptible to AMK, CST, CTX, and CXT. Thirty-eight (95%) strains were resistant to at least 1 antimicrobial tested, and all of these strains showed resistance to TET. Nineteen different antimicrobial resistance profiles were observed. MAR to more than 3 antibiotics was observed in 52.5% of the strains (Table 2).

Genotypic characterization and molecular subtyping of ETEC isolates

We observed 16 different virulence profiles among the 40 isolated ETEC strains (Table 3). ERIC-PCR analysis was

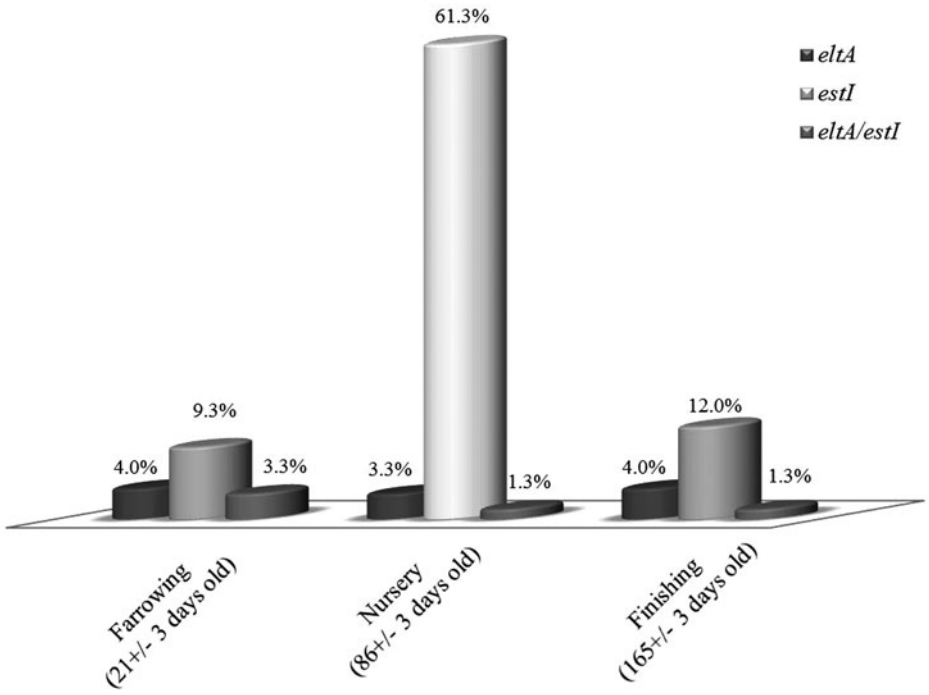

FIG. 1. Prevalence of *eltA* (toxin LT) and *estI* (toxin STa) genes obtained from the confluent growth zone in tryptic soy broth (TSB). A total of 90 rectal swabs were collected from each farm to determine the presence of *eltA* (toxin LT) and *estI* (toxin STa) genes and the subsequent prevalence of different genotypes in each production stage. Thirty randomly selected pigs, from three different stratums based upon the age and stage of production, were sampled.

able to subtype the 40 ETEC strains of different serotypes, generating 29 distinct ERIC-PCR patterns with 6–12 discernible fragments, ranging from 300 to 1500 bp. Sixteen isolates were grouped in 5 clusters (I–V), and 24 isolates presented unique ERIC-PCR patterns (Fig. 2). Cluster I included three ETEC strains, two of them belonging to serotype O157:H19 and one to serotype O157:H-. The Cluster I strains presented three different antimicrobial resistance patterns (TET, TET/FFN, and TET/NAL/FOF) and had three different genotypes (*rfb*$_{O157}$/*estII*/*aidA*, *rfb*$_{O157}$/*estII*/*aidA*/*astA*, and *rfb*$_{O157}$/*estII*). Cluster II grouped five O157:H19 strains that had four different antimicrobial resistance patterns (TET, TET/NAL, STR/TET/NAL, and TET/NIT/FOF) and one genotypic profile (*rfb*$_{O157}$/*estII*/*aidA*). Cluster III grouped four O157:H19 strains that had three different antimicrobial resistance profiles (TET, TET/FOF, and TET/SXT) and one genotype (*rfb*$_{O157}$/*estII*/*aidA*). Of the 12 strains grouped within Clusters I, II, and III, 11 strains were isolated from Farm 5 and 1 from Farm 4. Cluster IV included two O8:H31 strains from Farm 9 that had two antimicrobial resistance patterns (TET and TET/NAL) and presented two different genotypic patterns (*estII*/*astA* and *estII*/*astA*/*aidA*). Cluster V included two strains isolated from Farms 3 and 6, O8:H- and ONT:H-. These strains had two antimicrobial resistance patterns (AMP/TET/SXT and STR/TET/NAL/CIP/CHL/FFN) and two genotypic profiles (*estI*/*estII*/*intI2* and *estI*/*estII*).

Discussion

In pigs, ETEC is normally associated with ND and PWD; however, this pathogen can also be shed in feces from healthy animals (Osek, 1999). This study revealed the presence of ETEC in nondiarrheic pigs in different production stages. The overall prevalence of animals carrying *eltA*, *estI*, or both was 15.2%, which appears to be lower than that reported in animals with clinical diarrhea (Vidotto *et al.,* 2009; Zajacova *et al.,* 2012). In this study, the percentage of ETEC^{+} nondiarrheic piglets during the lactation period was 16.6%. Reported prevalence values in clinically affected neonates are higher, varying from 25.7% to 43.0% (Nakazawa *et al.,* 1987; Do *et al.,* 2006). However, the prevalence of ETEC^{+} nursery pigs observed during this study does not differ from other reports involving animals with clinical diarrhea (Fairbrother *et al.,* 2005; Do *et al.,* 2006). The prevalence of ETEC^{+} pigs observed during the finisher period is consistent with the prevalence of ETEC previously reported among animals at slaughter (Martins *et al.,* 2010). ETEC clinical manifestation is a complex process that not only requires the presence of ETEC strains (Dewey *et al.,* 1995) but also environmental changes, considered risk factors for the presence of clinical disease (Amezcua *et al.,* 2002; Laine *et al.,* 2008). Therefore, detection of subclinical carriers should be considered in order to prevent clinical manifestation and further dissemination of ETEC strains.

ETEC-STa strains are believed to be responsible for most cases of diarrhea in animals less than a week old; in animals more than 4 weeks old, diarrhea is more likely the result of ETEC-STb^{+} or ETEC-STb/a^{+} (Moon *et al.,* 1986). Although the *estII* gene was not evaluated in this study's screening stage, the genotypic characterization of ETEC strains showed *estII*'s prevalence at 39/40 (97.5%). Several studies have demonstrated that bacteria encoding *estII,* either alone or in combination with other toxins, are highly prevalent in

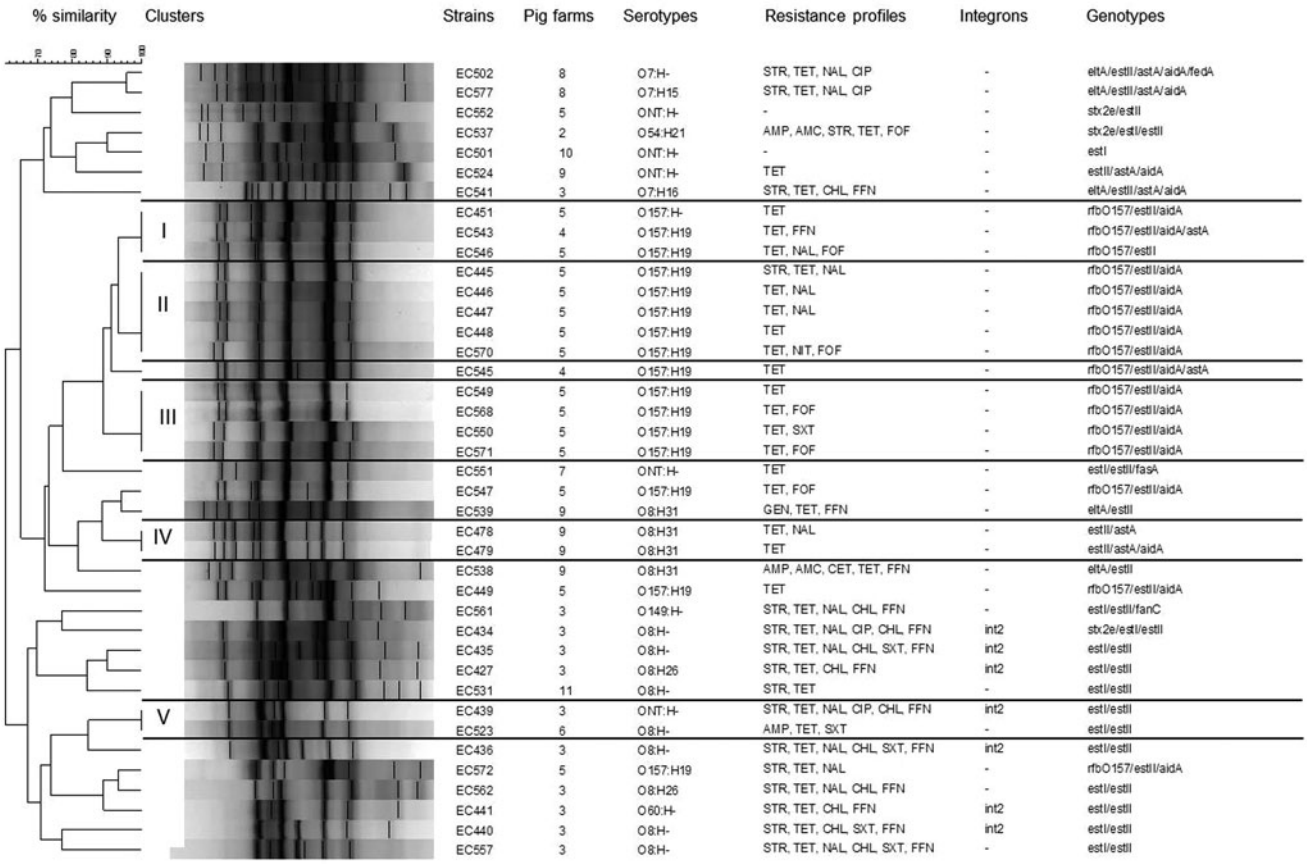

FIG. 2. Enterobacterial repetitive intergenic consensus–polymerase chain reaction (ERIC-PCR) dendrogram showing the genetic relationship, serotypes, antimicrobial resistance profiles (for antimicrobial abbreviations, see text), and virulence genes of Enterotoxigenic *Escherichia coli* (ETEC) isolated from nondiarrheic pigs. The dendrogram, generated by BioNumerics software (Applied Maths, Belgium), shows distances calculated by the Dice similarity index of ERIC-PCR among 40 ETEC strains. The degree of similarity (%) is shown on the scale. Roman numbers indicate cluster number. The dendrogram was generated based on the unweighted-pair group method with the arithmetic mean.

animals with clinical diarrhea (Dubreuil, 1997). In this study, the most frequent gene combination was rfb_{O157}/*estII*/*aidA* (32.5%). Previous reports proposed that ETEC-STb strains might lack an adherence factor, or there might be dysregulation of the amount of toxin produced. ETEC-STb is also commonly associated with the presence of F4 fimbria (Dubreuil 1997). In this study, the *estII* gene was present in ETEC isolates carrying F5, F6, and F18 genes, but not F4. Although F5 and F6 are commonly associated with ND, and F18 with PWD, we observed strains carrying genes encoding *estII* and fimbrial adhesins F5, F6, and F18 in nondiarrheic pigs. In this study, 8 strains (20.0%) harboring the *astA* gene were observed in association with ETEC-F6$^+$ and ETEC-F18$^+$ strains. The role of the EAST1 toxin in swine colibacillosis has not been fully demonstrated. However, the *astA* gene encoding the EAST1 toxin is commonly found in ETEC isolates associated with PWD (Vu Khac *et al.,* 2006; Moredo *et al.,* 2012).

Two additional virulence factors, AIDA-I and Stx2e, are present in ETEC strains associated with diseased pigs (Ngeleka *et al.,* 2003; Wang *et al.,* 2011). The AIDA-I gene seems to be equally prevalent in fimbrial and nonfimbrial isolates (Zhang *et al.,* 2007); in this study, 50.0% of isolates were ETEC-*aidA*$^+$; however, only a single isolate (*eltA*/*estII*/*astA*/*aidA*/*fedA*) was associated with the presence of an F18 gene. Previous reports suggested that the STb/AIDA association could be the missing link in what we know about swine colibacillosis; the similarities between AIDA-I of human origin and that of porcine origin could represent a potential danger of cross-infection between humans and pigs (Dubreuil, 2010). We observed three ETEC strains carrying stx_{2e}/*estI* and stx_{2e}/*estII*/*estII* genes. Our findings concur with recent studies that have observed an ETEC strain carrying stx_2 genes (Wang *et al.,* 2011). These strains carrying ETEC and Shiga toxin–producing *Escherichia coli* (STEC) genes have been proposed as ETEC/STEC hybrids carrying stx_2 gens (Tozzoli *et al.,* 2014). The importance of these findings is due to the potential risk of human infection with ETEC/STEC hybrids (Wester *et al.,* 2013).

Previous studies have observed, in varying frequency, the following serogroups associated with PWD and ED: O8, O45, O138, O139, O141, O149, and O157 (Frydendahl, 2002; Fairbrother *et al.,* 2005). Fairbrother *et al.* (2005) have described serogroups O149 and O157 as the most frequent cause of ND, PWD, and ED. In the present study, serotypes O8:[H21,H26,H31], O149:H-, and O157:H19 were the most prevalent among ETEC isolates in nondiarrheic pigs. The serogroups detected in this study concur with those reported in the literature as causative agents of disease in pigs

Table 2. Antimicrobial Resistance Profile and *intI2* Gene of Enterotoxigenic *Escherichia coli* Strains from Nondiarrheic Pigs

Resistance pattern	*Pig farms*	*Resistance profile*[a]	*Isolates* N *(%)*	*Integrons (*N*)*
1	5, 4, 7, 9	TET	8 (20.0)	
2	5, 9	TET, NAL	3 (7.5)	
3	5	TET, FOF	3 (7.5)	
4	3	STR, TET, CHL, FFN^{MAR}	3 (7.5)	*intI2* (2)
5	3	STR, TET, NAL, CHL, SXT, FFN^{MAR}	3 (7.5)	*intI2* (2)
6	5	STR, TET, NAL^{MAR}	2 (5.0)	
7	8	STR, TET, NAL, CIP^{MAR}	2 (5.0)	
8	3	STR, TET, NAL, CHL, FFN^{MAR}	2 (5.0)	
9	3	STR, TET, NAL, CIP, CHL, FFN^{MAR}	2 (5.0)	*intI2* (2)
10	4	TET, FFN	1 (2.5)	
11	5	TET, SXT	1 (2.5)	
12	11	STR, TET	1 (2.5)	
13	5	TET, NIT, FOF^{MAR}	1 (2.5)	
14	6	AMP, TET, SXT^{MAR}	1 (2.5)	
15	5	TET, NAL, FOF^{MAR}	1 (2.5)	
16	9	GEN, TET, FFN^{MAR}	1 (2.5)	
17	9	AMP, AMC, CET, TET, FFN^{MAR}	1 (2.5)	
18	2	AMP, AMC, STR, TET, FOF^{MAR}	1 (2.5)	
19	3	STR, TET, CHL, SXT, FFN^{MAR}	1 (2.5)	*intI2*

[a]Antimicrobial: ampicillin (AMP), cephalothin (CET), amoxicillin/clavulanic acid (AMC), gentamicin (GEN), streptomycin (STR), tetracycline (TET), nalidixic acid (NAL), ciprofloxacin (CIP), chloramphenicol (CHL), florfenicol (FFN), trimethoprim-sulfamethoxazole (SXT), nitrofurantoin (NIT), fosfomycin (FOF).
[MAR]Multiple antimicrobial resistances.

(Frydendahl, 2002; Fairbrother *et al.,* 2005). Serotypes O157:H7 and O8:[H2,H8,H9,H19,H-] have been identified in human outbreak of STEC (Friesema *et al.,* 2015; Kaper and O'Brien, 2014). Although only Shiga toxin–negative strains of *E. coli* O157 has been proved to be capable of acquiring *stx* genes (Wetzel and LeJeune, 2007), the potential role of Shiga toxin–negative strains of *E. coli* O8 cannot be ruled out, increasing their potential to emerge as new Shiga toxin–producing *E. coli* strains.

Table 3. Virulence Genes and Integrons Profiles of Enterotoxigenic *Escherichia coli* (ETEC) Isolated from Nondiarrheic Pigs

Genotype	n *(%)*
*rfb*O157/*estII/aidA*	13 (32.5)
estI/estII/intI2	6 (15.0)
estI/estII	4 (10.0)
*rfb*O157/*estII/aidA/astA*	2 (5.0)
eltA/estII	2 (5.0)
estII/astA/aidA	2 (5.0)
eltA/estII/astA/aidA	2 (5.0)
*rfb*O157/*estII*	1 (2.5)
estI/estII/fasA	1 (2.5)
estII/astA	1 (2.5)
eltA/estII/astA/aidA/fedA	1 (2.5)
stx_{2e}/*estII**	1 (2.5)
stx_{2e}/*estI/estII/intI2**	1 (2.5)
stx_{2e}/*estI/estII**	1 (2.5)
estI	1 (2.5)
estI/estII/fanC	1 (2.5)

ETEC/Shiga toxin–producing *Escherichia coli* hybrids* (n): number of strain.

Of the 40 ETEC strains, 16 (40.0%) were characterized as O157:[H19/H-]. According to the subtyping analysis performed in this study, ETEC O157:[H19/H-] strains were grouped in three different clusters (I, II, and II); however, these strains differed by antimicrobial resistance patterns and genotypic profile. These results are consistent with previous reports in which ERIC-PCR had a low discriminatory rate among strains of *E. coli* O157:H7 (Giammanco *et al.,* 2002). However, we observed that ERIC-PCR has better discriminatory power among ETEC O8:[H26/H31/H-] strains, the second most prevalent serotype in our study. These isolates were grouped into two clusters, and the technique was capable of differentiating nine strains with individual ERIC-PCR patterns.

Previous publications demonstrated that *E. coli* strains responsible for PWD could be resistant to multiple antibiotics (Fairbrother *et al.,* 2005; Wang *et al.,* 2011). In our study, 52.5% of the strains showed different MAR patterns. Prophylactic uses of antibiotics seem to play a role in MAR. A small number of studies have demonstrated that certain groups of drugs can have a prophylactic effect; however, when these antibiotics were withdrawn, clinical cases of PWD increased (Casewell *et al.,* 2003). In this study, 7 (17.5%) of the ETEC strains were positive for *intI*2 and resistant to STR, TET, CHL, and FFN. Previous studies have shown an association between MAR strains, including STR and TET in their multiresistance profiles, and the presence of Class 2 integrons detected in *E. coli* strains isolated from swine (Kadlec and Schwarz, 2008; Lapierre *et al.,* 2008). The presence of MARs ETEC strains suggest that there is a great need for surveillance programs to monitor MARs *E. coli* that can be potentially transmitted to humans from food animals.

This study demonstrates that numerous potentially virulent ETEC genotypes cohabit and circulate in swine populations without clinical manifestation of ND or PWD. The information generated is important not only for diagnostic and epidemiological purposes, but also for understanding the dynamics and ecology of ETEC in pigs in different production stages that can be potentially transmitted to humans from food animals.

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Address correspondence to:
Pablo E. Piñeyro, DVM
Department of Veterinary Diagnostic and Production Animal Medicine
College of Veterinary Medicine
Iowa State University
1655 Veterinary Medicine
Ames, IA 50011

E-mail: pablop@iastate.edu