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Diverse Virulence Gene Content of Shiga Toxin-Producing *Escherichia coli* from Finishing Swine

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Shiga toxin-producing *Escherichia coli* (STEC) infections are a critical public health concern because they can cause severe clinical outcomes, such as hemolytic uremic syndrome, in humans. Determining the presence or absence of virulence genes is essential in assessing the potential pathogenicity of STEC strains. Currently, there is limited information about the virulence genes carried by swine STEC strains; therefore, this study was conducted to examine the presence and absence of 69 virulence genes in STEC strains recovered previously from finishing swine in a longitudinal study. A subset of STEC strains was analyzed by pulsed-field gel electrophoresis (PFGE) to examine their genetic relatedness. Swine STEC strains ($n = 150$) were analyzed by the use of a high-throughput real-time PCR array system, which included 69 virulence gene targets. Three major pathotypes consisted of 16 different combinations of virulence gene profiles, and serotypes were determined in the swine STEC strains. The majority of the swine STEC strains ($n = 120$) belonged to serotype O59:H21 and carried the same virulence gene profile, which consisted of 9 virulence genes: *stx*$_{2e}$, *iha*, *ecs1763*, *lpfA*$_{O113}$, *estIa* (*STa*), *ehaA*, *paa*, *terE*, and *ureD*. The *eae*, *nleF*, and *nleH1-2* genes were detected in one swine STEC strain (O49:H21). Other genes encoding adhesins, including *iha*, were identified ($n = 149$). The PFGE results demonstrated that swine STEC strains from pigs raised in the same finishing barn were closely related. Our results revealed diverse virulence gene contents among the members of the swine STEC population and enhance understanding of the dynamics of transmission of STEC strains among pigs housed in the same barn.

Shiga toxin-producing *Escherichia coli* (STEC) infections are a critical public health concern, leading to 170,000 cases of human illness (1) and an economic burden of 280 million dollars (2) annually in the United States. STEC strains represent a subset of *E. coli* strains that produce one or more bacteriophage-encoded cytotoxins known as Shiga toxins (Stx1 and Stx2) (3, 4). Enterohemorrhagic *E. coli* (EHEC) strains represent a subgroup of STEC strains defined by the presence of an Stx-encoding bacteriophage and the presence of the locus of enterocyte effacement (LEE) pathogenicity island, which is important for the development of attaching and effacing lesions (5). The LEE island is not present in all STEC strains. Prior studies have found that EHEC is more commonly associated with severe clinical cases (6); however, LEE-negative STEC strains have been linked to severe clinical cases as well as to outbreaks (7). STEC is often acquired by consuming contaminated food or water (8), and cattle are viewed as the most important animal reservoirs (9). Food of bovine origin has been implicated in many STEC infections and outbreaks, though a number of other food products, including pork products, have been confirmed as the source of STEC in a number of outbreaks (10–16). For example, a recent STEC O157:H7 outbreak was associated with consuming large cuts of pork from a whole roasted pig (16).

Although the way in which the pork products became contaminated in these outbreaks was uncertain (10–16), the likelihood that on-farm pigs were the source of STEC contamination cannot be overlooked. Unlike cattle, which do not usually present clinical symptoms due to STEC infection, pigs, specifically postweaning and young finishing pigs, can suffer from edema disease caused by STEC strains carrying the *stx*$_{2e}$ variant (17). Epidemiological studies conducted in different regions of the world have reported a wide range of STEC prevalences in swine populations (18–20). However, the epidemiology and virulence characteristics of STEC carried by on-farm pigs remain largely unknown, as does whether swine-derived STEC strains are similar to human-derived STEC strains and have the potential to contribute to human infections.

Human STEC infections are associated with a range of clinical symptoms: diarrhea, hemorrhagic colitis (HC), and the life-threatening hemolytic uremic syndrome (HUS) (6, 21). The pathogenesis of STEC in human patients has been reviewed elsewhere (9, 22–26). Although Shiga toxins are critical in STEC pathogenesis because they inhibit host cell protein synthesis (27) and induce apoptosis (reviewed in reference 28), virulence factors other than Stx are also important (29). For example, following initial attachment to host intestinal cells, EHEC strains that express intimin, which is encoded by *eae* on the LEE pathogenicity island, can intimately attach to host cells (23, 30). The N-terminal region of intimin is highly conserved, whereas the C-terminal region is variable and accounts for the definition of *eae* subtypes. For STEC strains that lack *eae* (LEE-negative STEC) and cause diseases in humans, other adherence structures have been suggested to be important. For example, the STEC autoagglutinating adhesin encoded by *saa* increases adherence of the pathogen to human epi-

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thelial cells (31). Other structures contributing to STEC colonization, such as the long polar fimbriae encoded by *lpf*, which has many variants, have also been found in both EHEC strains and LEE-negative STEC strains (23, 32, 33). A number of other virulence factors have been proposed to contribute to STEC pathogenesis, for instance, catalase peroxidase encoded by *katP*, which protects STEC from peroxide-mediated oxidative damage (34). Some non-LEE-carried effector (*nle*) genes, which encode proteins having various functions, such as inhibiting phagocytosis, have been detected in EHEC strains isolated from human patients with severe clinical disease, namely, HC or HUS (29, 35, 36).

Knowing that various combinations of virulence factors contribute to STEC pathogenesis, it is essential to determine the presence or absence of specific virulence genes to better assess the potential pathogenicity of STEC strains (29, 32, 37). A few studies have examined the virulence gene profiles of swine STEC strains (38–42). However, every study selected different panels of virulence genes, which makes comparison of results across different studies highly challenging. The presence of many virulence genes, for example, *nle* genes and allelic variants, has seldom been examined in swine STEC strains. In general, little is known about the virulence characteristics of STEC originating from swine that may contribute to disease in humans and swine. To fill in the current knowledge gap and better evaluate the potential pathogenicity of swine-derived STEC strains, we utilized a PCR microarray method to examine the presence of 69 virulence genes in STEC strains recovered previously from finishing pigs in a longitudinal study (43). Moreover, the genetic relatedness of these strains was also examined to better understand STEC transmission dynamics in swine throughout the finishing period.

MATERIALS AND METHODS

Swine STEC strains. A total of 150 STEC strains recovered from 95 finishing pigs in a longitudinal study were included in this study (43). Individual fecal samples were collected from three cohorts of finishing pigs ($n = 50$/cohort, $n = 150$ in total). Each cohort was raised in a separate finishing barn at two finishing sites (cohort 1 at site A, cohorts 2 and 3 at site B) within one all-in, all-out multisite production system in the midwestern United States. The samples in each cohort were collected every 2 weeks during the finishing period (8 farm visits/cohort). A sample was considered to be positive for STEC when an STEC isolate was recovered. The presence of virulence genes (stx_1, stx_2, stx_{2e}, and *eae*) and the O:H serotypes were determined in these STEC isolates (43). At least one STEC strain was selected from each positive sample for virulence gene characterization by PCR microarray (see below). STEC strains belonging to different serotypes recovered from the same sample were also included in the study to better understand the diversity of STEC strains within the animals.

Selection of virulence gene targets. The general rationale for the selection of virulence gene targets was based on their function, role in pathogenesis, and association with human illness and/or disease severity in human patients. According to the previous characterization results, only 1 among the 150 swine STEC strains carried the *eae* gene (43). Additionally, all swine STEC strains recovered in the longitudinal study were in non-O157 serogroups (43). Therefore, the 69 virulence genes targeted in the microarray were selected based on genes found in different STEC serogroups and included putative genes encoding adhesins (*iha*, *paa*, *orfA*, *orfB*, *toxB*, *eibG*, *saa*, *eae*, and allelic variants), toxins {*stx* [all variants, including stx_{2e}], *ent* (*espL2*), *cdtI*, *cdtIII*, *astA*, *estIa* (*STa*), *elt* (*LT*), and *subAB*}, fimbriae ($lpfA_{O157}$, $lpfA_{O113}$, $lpfA_{O26}$, and *sfp*), and others found in pathogenic STEC strains [*terE*, *ureD*, *espV*, *espK*, *espN*, *espX7*, *espO1-1*, *nleG5*, *nleG6-2*, *Z2096*, *Z2098*, *Z2099*, *nleA*, *nleF*, *nleH1-2*, *espM1*, *espM2*, *nleB*, *nleE*, *efa1* (*lifA*), *pagC*, *ecs1822*, *ecs1763*, *irp2*, *fyuA*, *ehaA*, *hlyA*, *bfp*, *cnf2*, *ecf1*, *ecf2*, *ecf3*, *efc4*, *katP*, *ehxA*, *etpD*, *stcE*, *espP*, *epeA*, and *sab*]. Some of the virulence genes were also those associated with swine diseases: postweaning diarrhea [*estIa* (*STa*), *elt* (*LT*), *orfA*, *orfB*, and *hlyA*] and edema disease (stx_{2e}, *orfA*, *orfB*, and *hlyA*). We also included fimbrial genes that are associated with swine neonatal diarrhea [*fasA* (F6) and *fimF41a* (F41)], postweaning diarrhea [*fedA* (F18)], and swine edema disease [*fedA* (F18)].

High-throughput real-time PCR microarray. The BioMark real-time PCR system (Fluidigm) was used for high-throughput microfluidic real-time PCR amplification using 96.96 dynamic arrays (Fluidigm). Amplifications were performed using EvaGreen DNA binding dye (Biotium Inc., Hayward, CA) with TaqMan Gene Expression master mix in accordance with the recommendations of the manufacturer (Applied Biosystems, Courtaboeuf, France). The thermal profile comprised 10 min at 95°C, followed by 35 cycles of 95°C for 15 s and 60°C for 1 min, followed by a melting-curve analysis.

Besides the 69 selected virulence genes, O-group-associated genes specific for serogroups O26, O157, O145, O103, O111, O121, O45, O118, O128, O146, O91, O104, O113, and O55 were included in the PCR microarray. Moreover, the microarray chip also targeted flagellar genes for H-groups H11, H7, H21, H2, H28, H8, H19, H16, H25, H4, and H32 (44). Most of the primers used in the PCR microarray were described previously (29, 38, 44, 45), and some were designed for this study. The sequences of all primers are reported in Table S1 in the supplemental material.

Strain selection strategy for PFGE. A subset of swine STEC strains ($n = 49$) was selected for pulsed-field gel electrophoresis (PFGE) analysis to determine their genetic relatedness. The selection criteria were based on serotype, virulence gene profiles, and epidemiological information related to the pigs. Within the predominant serotype, O59:H21, strains recovered in the early, middle, and late stages of the finishing period were selected. We included STEC strains of the same serotype and recovered from the same pig at different farm visits over the finishing period to examine changes over time. STEC strains of the same serotype but with different virulence gene profiles were also selected for PFGE analysis. In total, 29 O59:H21 STEC strains were selected, as well as 2 O59:H19 STEC strains. Thirteen additional STEC strains belonging to serotype O untypeable: H19, four O98:H12 STEC strains, and one O98:H19 strain were also analyzed.

PFGE. PFGE was conducted according to the standardized Centers of Disease Control and Prevention (CDC) PulseNet protocol (46). In summary, STEC DNA was embedded in agarose and digested with 50 U of XbaI for 2 h at 37°C. A CHEF DR-III system (Bio-Rad, Munich, Germany) was used to separate the restriction fragments by electrophoresis at pulse times of 2.16 to 54.17 s in 0.5× Tris-borate-EDTA buffer with 50 μM thiourea at 14°C for 16.2 h. The H9812 *Salmonella enterica* serovar Braenderup strain (CDC, Atlanta, GA) was utilized as a molecular size marker. BioNumerics software package 6.6 (Applied Maths, Ghent, Belgium) was used to analyze the PFGE restriction-digested band patterns. The dendrogram was built by analyzing Dice coefficients and by using the unweighted-pair group method using average linkages (UPGMA) with 0.5% band position tolerance. The genetic relatedness of the strains was assessed by the percentages of similarity of the PFGE patterns.

RESULTS

Virulence gene profiles of swine STEC strains. There were 11 distinct virulence gene profiles among the swine STEC strains tested in this study, with 16 different combinations based on serotype and virulence gene profiles (Table 1). The strains had between 6 and 20 genes among the 69 virulence genes examined, and most strains (82% [123/150]) carried the same virulence gene profile (virulence gene profile 1), which consisted of the following 9 genes: stx_{2e}, *iha*, *ecs1763*, $lpfA_{O113}$, *estIa* (*STa*), *ehaA*, *paa*, *terE*, and *ureD*. The second-most-prevalent virulence gene profile was profile 3, found in 10% (15/150) of the strains, and contained stx_{2e},

TABLE 1 Distribution of swine STEC strains by serotype and virulence gene profiles

Serotype	No. of swine STEC strains	No. of strains analyzed by PFGE	Virulence gene profile	Virulence gene profile code	Pathotype
O59:H21	118	27	*stx*$_{2e}$, *iha*, *ecs1763*, *lpfA*$_{O113}$, *estIa* (*STa*), *ehaA*, *paa*, *terE*, *ureD*	1	II
	2	2	*stx*$_{2e}$, *iha*, *ecs1763*, *lpfA*$_{O113}$, *ehaA*, *paa*, *terE*, *ureD*	2	I
O untypeable:H19	13	12	*stx*$_{2e}$, *iha*, *estIa* (*STa*), *paa*, *terE*, *ureD*	3	I
	1	1	*stx*$_{2e}$, *iha*, *astA*, *estIa* (*STa*), *terE*, *ureD*	4	III
O59:H19	2	2	*stx*$_{2e}$, *iha*, *estIa* (*STa*), *paa*, *terE*, *ureD*	3	I
O98:H12	2	2	*stx*$_1$, *pag C*, *katP*, *iha*, *astA*, *ecf1*, *ecf2*, *ecf3*, *ecf4*, *paa*, *terE*, *ureD*	9	III
	2	2	*stx*$_1$, *pag C*, *katP*, *iha*, *ecf1*, *ecf2*, *ecf3*, *ecf4*, *paa*, *terE*, *ureD*	10	III
O untypeable:H21	2	0	*stx*$_{2e}$, *iha*, *ecs1763*, *lpfA*$_{O113}$, *estIa* (*STa*), *ehaA*, *paa*, *terE*, *ureD*	1	II
O20:H21	1	0	*stx*$_{2e}$, *iha*, *ecs1763*, *lpfA*$_{O113}$, *estIa* (*STa*), *ehaA*, *paa*, *terE*, *ureD*	1	II
O49:H21	1	0	*stx*$_{2e}$, *eae*, *nleF*, *nleH1-2*, *katP*, *iha*, *ecs1763*, *lpfA*$_{O113}$, *astA*, *estIa* (*STa*), *ecf1*, *ecf2*, *ecf3*, *ecf4*, *irp2*, *fyuA*, *ehaA*, *paa*, *terE*, *ureD*	11	III
O89:H19	1	0	*stx*$_{2e}$, *iha*, *lpfA*$_{O113}$, *estIa* (*STa*), *ehaA*, *paa*, *terE*, *ureD*	7	I
O98:H19	1	1	*stx*$_{2e}$, *pagC*, *katP*, *iha*, *ecf1*, *ecf2*, *ecf3*, *ecf4*, *paa*, *terE*, *ureD*	5	I
O115:H19	1	0	*stx*$_{2e}$, *iha*, *ecs1763*, *lpfA*$_{O113}$, *astA*, *estIa* (*STa*), *orfA*, *orfB*, *ehaA*, *paa*, *terE*, *ureD*	6	II
O119:H21	1	0	*stx*$_{2e}$, *iha*, *ecs1763*, *lpfA*$_{O113}$, *estIa* (*STa*), *ehaA*, *paa*, *terE*, *ureD*	1	II
O167:H21	1	0	*stx*$_{2e}$, *iha*, *ecs1763*, *lpf A*$_{O113}$, *estIa* (*STa*), *ehaA*, *paa*, *terE*, *ureD*	1	II
O untypeable:H4	1	0	*stx*$_{2e}$, *fedA* (F18), *hlyA*, *orfA*, *orfB*, *paa*, *terE*	8	III
Total	150	49			

iha, *estIa* (*STa*), *paa*, *terE*, and *ureD*. One strain, serotype O49:H21, carried virulence gene profile 11, which included *eae* and two *nle* variants (*nleF* and *nleH1-2*) as well as *stx*$_{2e}$, *katP*, *iha*, *ecs1763*, *lpfA*$_{O113}$, *astA*, *estIa* (*STa*), *ecf1*, *ecf2*, *ecf3*, *ecf4*, *irp2*, *fyuA*, *ehaA*, *paa*, *terE*, and *ureD*. Furthermore, the data showed that the strains can be grouped into three major pathotypes according to the virulence genes they carried (Table 1). Pathotype I was defined as the strains (12.7% [19/150]) possessing 5 core genes, including *stx*$_{2e}$, *iha*, *paa*, *terE*, and *ureD*, along with other virulence genes. In addition to the 5 core genes, pathotype II strains (82.7% [124/150]) possessed the 4 genes *ecs1763*, *estIa* (*STa*), *lpfA*$_{O113}$, and *ehaA* along with other virulence genes. Finally, pathotype III strains carried *stx*$_1$ (2.7% [4/150]) and *eae* (0.7% [1/150]), as well as other virulence genes (1.3% [2/150]).

Although only 1 of the 150 strains had *eae*, other genes encoding proteins associated with attachment were present in the swine STEC strains. For example, *iha*, which encodes the iron-regulated gene A homolog adhesin (47), was detected in 99.3% (149/150) of the STEC strains. The *lpfA*$_{O113}$ gene, which encodes long polar fimbriae (48), was detected in 85.3% (128/150) of the strains, and the *fedA* gene, which encodes fimbrial adhesin F18 and is associated with swine edema disease and postweaning diarrhea (49), was present in 0.7% (1/150) of the strains. The *orfA* and *orfB* genes, which encode adhesins involved in diffuse adherence (AIDA) (50, 51), were present in 1.3% (2/150) of the strains. Moreover, the *paa* gene, which encodes the porcine attaching and effacing-associated adhesin (52), was detected in 99.3% (149/150) of the strains. Among the fimbrial genes (*fimF41a* and *fasA*), which contribute to colonization in swine and are associated with swine neonatal diarrhea (17), *fimF41a* was detected in 0.6% (1/150) of the strains, while *fasA* was not detected in any of the strains.

A number of genes that encode toxins and hemolysins were present in the panel of swine STEC strains. For instance, 146 of the 150 swine STEC strains carried the *stx*$_{2e}$ gene, and the other four strains carried *stx*$_1$. The *astA* gene, which encodes the enteroaggregative *E. coli* (EAEC) heat-stable toxin (53), was detected in 3.3% (5/150) of the strains. The *estIa* (*STa*) gene, which encodes heat-stable toxin (54), was detected in 94.7% (142/150) of the strains. Moreover, the *hlyA* gene, which encodes the alpha hemolysin (55), was present in 0.6% (1/150) of the strains. Interestingly, the *ecs1763* gene, which encodes hypothetical proteins and was previously detected in EHEC strains (56), was present in 84.7% (127/150) of the strains. The following virulence genes, which were also targeted in the PCR microarray, were not detected in any of the 150 swine STEC strains: *eae* subtypes alpha, beta, gamma, epsilon, and theta, *nleA*, *nleG5*, *ent* (*espL2*), *nleB*, *nleE*, *efa1* (*lifA*), *Z2096*, *Z2098*, *Z2099*, *espM1*, *espM2*, *nleG6-2*, *espK*, *espN*, *espX7*, *espO1-1*, *espV*, *ecs1822*, *sfp*, *bfp*, *lpfA*$_{O26}$, *lpfA*$_{O157}$, *cdt* subtypes I and III, *elt* (*LT*), *cnf2*, *ehxA*, *toxB*, *stcE*, *eibG*, *epeA*, *espP*, *saa*, *subAB*, and *sab*.

PFGE. Within the O59:H21 STEC strains, three major clusters (clusters A to C) were defined at a cutoff value of 80% similarity (Fig. 1). These three major clusters were related at 71.1% similarity. The strains isolated from pigs within the same cohort were clustered. For example, cluster A contained strains from pigs in cohort 3, and cluster B contained strains from pigs in cohort 1. The only exception was that strain 297 from pig 119 in cohort 3 clustered with strains from cohort 2 (cluster C). Strains from pigs in cohort 3 (cluster A) were related to the strains from pigs in cohort 1 (cluster B) at 75.1% similarity. Within each cluster, indistinguishable PFGE patterns were observed among STEC strains recovered from samples in the same pig over time during the finishing period. For example, strains 170 and 228 with indistinguishable PFGE patterns were recovered from pig 145 at the second and third farm visits in cohort 3. In cluster A, strains carrying two different virulence gene profiles (profiles 1 and 2) clustered. The two O59:H19 strains, which carried virulence gene profile 3, were not clustered with the O59:H21 strains (24.8% similarity; data not shown).

Within the O untypeable:H19 strains, 11 of the 13 strains were clustered at 83.6% similarity. One of the O untypeable:H19 strains had a PFGE pattern different from those of the other 11 strains

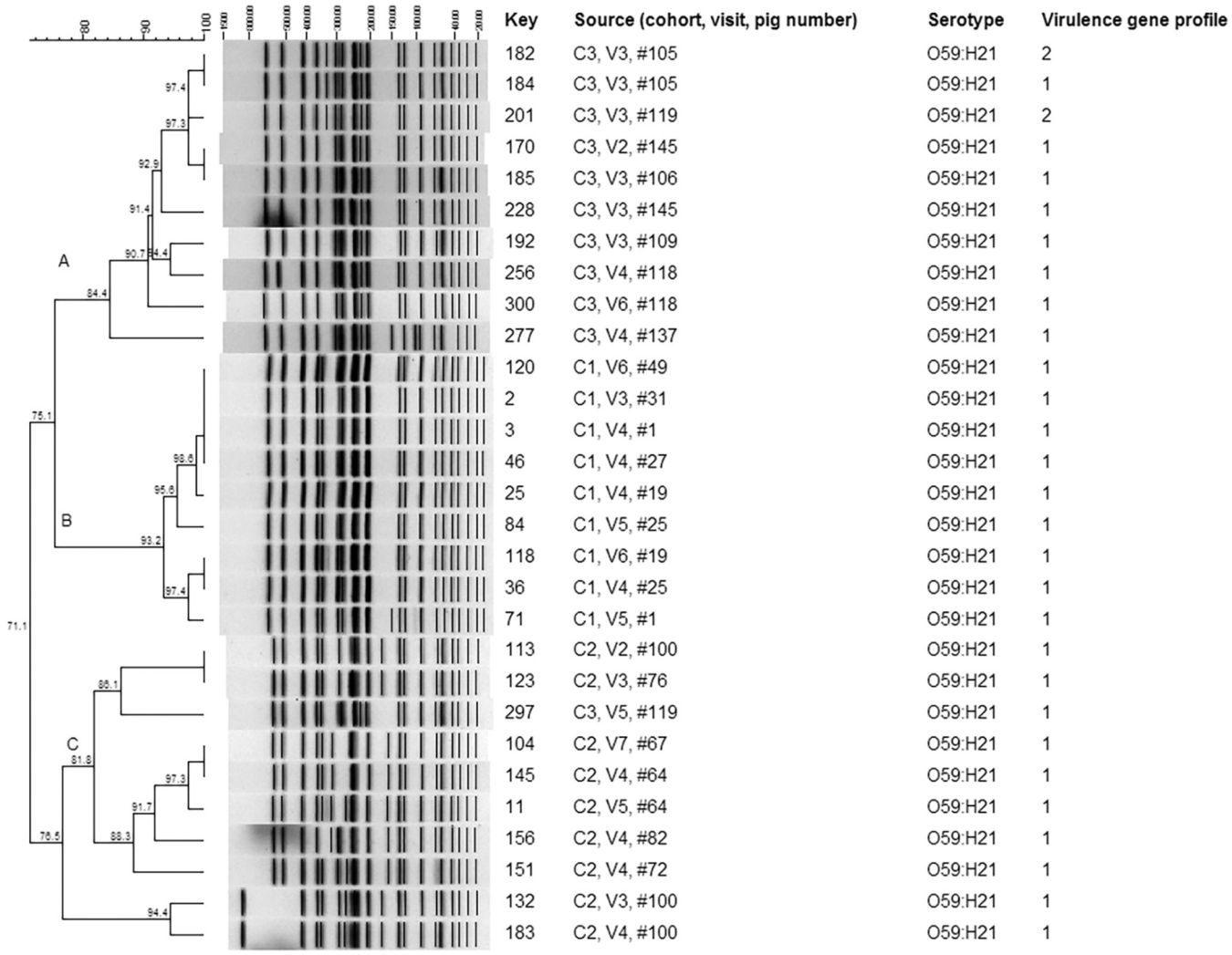

FIG 1 PFGE analysis of swine O59:H21 STEC strains. The "Key" column lists strain numbers. The "Source" column lists the cohort number of the pig from which each strain was collected, the number corresponding to the visit (of the eight farm visits) during which the strain was collected, and the individual pig number.

(27.4% similarity). Moreover, strain 281, which carried a different virulence gene profile, was not clustered with the other 12 strains (see Fig. S1 in the supplemental material). Within the five strains belonging to serogroup O98, the four O98:H12 strains had indistinguishable PFGE patterns and were clustered at a similarity level of over 90%. The O98:H19 strain ($n = 1$) did not cluster with the four O98:H12 strains (see Fig. S2 in the supplemental material).

DISCUSSION

The objective of this study was to use molecular methods to characterize swine STEC strains recovered in a previous study (43) to determine their virulence gene profiles and genetic relatedness. This study utilized a high-throughput real-time PCR platform to examine the presence of members of a large panel of virulence gene targets in swine STEC strains. Although these swine STEC strains were recovered from samples of 95 healthy finishing pigs from three cohorts within 18 months in the same geographic area, they were composed of three major pathotypes with 16 different combinations of virulence gene profiles and serotypes. The panel of virulence genes in this study included 69 targets, and our results were in agreement with those of another study suggesting that increasing the number of virulence genes in the panel would increase the resolution of the virulence gene profiling (57). Various virulence gene profiles in swine STEC strains have also been reported elsewhere (38–42, 58–60). However, it was challenging to compare the results of this study with the results of those previous reports because all of the studies employed different panels of virulence genes. Taken together, our results and the results of previous studies of swine STEC strains have indicated that the swine STEC group consists of strains carrying diverse sets of virulence genes.

Because the swine STEC strains examined in this study represent non-O157 serotypes and because only 1 of the 150 strains carried *eae*, we chose to screen for the presence of many novel virulence gene targets previously reported in non-O157 and LEE-negative STEC strains. Some adhesin-encoding genes which have been detected in human-pathogenic STEC strains were present in the swine STEC strains. For example, *iha* and $lpfA_{O113}$ were pres-

ent in over 80% of the swine STEC strains. The *iha* gene has been detected in over 70% of the LEE-negative STEC strains associated with human clinical cases examined in studies in Germany (61) and Australia (7). Both *iha* and $lpfA_{O113}$ have been detected in over 80% of LEE-negative STEC strains associated with human clinical cases examined in a study in Argentina (33). In addition to their potential ability to allow STEC to attach to human cells, the high prevalence of these genes in swine STEC may also suggest a role in STEC colonization of swine or enhanced transmissibility and persistence within a farm. These results warrant future research to better define the role that these attachment proteins may play in adherence to both swine and human epithelial cells.

The majority of swine STEC strains in this study carried stx_{2e}, which is associated with edema disease in swine (9). Although stx_{2e} was prevalent in the STEC strains in this study, none of the pigs in this study presented clinical symptoms (43). One of the potential explanations for the absence of clinical symptoms in these pigs was that only one of the strains carried the important fimbrial adhesin gene that is associated with swine edema disease (*fedA*) (17). Moreover, the fimbrial adhesin gene associated with swine neonatal diarrhea (*fimF41a*) (17) was present in only one swine STEC strain examined in this study, and *fasA* was not detected in any of the strains. Similarly, a previous study reported a high prevalence of stx_{2e} (80%) and a low prevalence of *fedA* (4.6%) and did not detect *fimF41a* and *fasA* in STEC strains from clinically healthy pigs (38). The production of Stx alone, without an adherence factor, is deemed to be insufficient to cause severe disease. In addition, none of swine STEC strains in this study belonged to serogroups O138, O139, O141, and O147, which are associated with edema disease (17). Lastly, pigs in this study were in the finishing period (10 to 24 weeks old), which is later than the usual onset age for neonatal diarrhea (0 to 4 days old), postweaning diarrhea, and edema disease (5 weeks old) (17). In the case of edema disease, the expression of receptors for the STEC fimbrial adhesin (*fedA*) in pigs is associated with younger age (17). Therefore, the older age of pigs in this study may also explain why they did not develop clinical symptoms when infected with *E. coli* strains carrying stx_{2e}.

In addition to swine diseases, Stx2e-producing *E. coli* strains are often implicated in infections of humans with mild disease or no clinical symptoms (62–64). Stx variants are known to be associated with disease severity in humans. For example, the stx_{2c} and stx_{2d} activatable variants are more likely to be found in STEC strains from HUS patients (64, 65), while stx_1 variants are associated with STEC strains in humans with milder clinical symptoms (62–64). Therefore, the swine STEC strains in this study predominantly carrying stx_{2e} and a few with stx_1 may represent low risk to human health. Moreover, one may notice that some swine STEC strains analyzed in this study were O-untypeable and that most of those that were identified belonged to serotypes that have not previously been associated with human infections, except O59:H19 (33). Nevertheless, in some rare cases, Stx2e-producing *E. coli* strains have been recovered from HUS cases (66) and from humans with uncomplicated diarrhea (62, 63, 67, 68). More research is needed to characterize and examine the frequencies of different *stx* variant genes in swine-derived STEC strains from farms in different geographic locations.

Several of the gene targets assessed in this study have not been examined in swine STEC strains elsewhere. For example, the *ecs1763* gene, which was found only in a subset of EHEC strains analyzed in a previous study (56), was prevalent in a high proportion (84.7%) of the swine strains. However, the function associated with this *ecs1763* gene has not yet been determined, and the association between the presence of this gene and the clinical outcome in human cases requires more research. In addition, the combination of *espK* with *espV*, *ureD*, or *Z2098* has been suggested to be highly prevalent in EHEC strains and can be utilized for EHEC detection purposes (45). Although *ureD* was present in 99.3% of our strains, the *espK*, *espV*, and *Z2098* genes were absent in the panel of strains used in this study. Considering the uncertainties of the role of these putative virulence factors in causing human illness, it is difficult to determine the health risk of many of these swine STEC strains.

Our study is the first one to use PFGE to analyze STEC strains recovered from repeated samples collected from pigs, while most of the previous studies used PFGE to determine the genetic relatedness of STEC strains from swine and other species (60, 69–72). The degrees of genetic relatedness of swine STEC strains to strains from other animal species differed in those studies, and most studies focused on STEC O157:H7 (60, 69–71). Here, we found that STEC O59:H21 strains, which predominated in this swine population, were closely related among pigs in the same cohort. This suggests that the same strain disseminated throughout each cohort and provides support for the idea of a point-source outbreak at each of the three barns at two distinct finishing sites (43). Thus, the pigs may have been exposed to the same point source of infections in the finishing-site environment. A longitudinal follow-up study in cattle also reported closely related PFGE patterns among STEC strains from cattle in the same cohort on the same farm (73). More research, however, is needed to identify potential risk factors for STEC shedding in swine and the common source of infection associated with STEC strains shed by finishing swine.

It was found that swine STEC strains of the same serotype could carry different virulence gene profiles. For example, two distinct virulence gene profiles (profiles 1 and 2) were identified within O59:H21 strains; however, the strains were closely related by PFGE. This was not unexpected, as the two virulence gene profiles (profiles 1 and 2) differed by the presence or absence of *estIa* (*STa*), which is carried on a plasmid that can readily be passed between strains with similar genetic backgrounds (54). Because PFGE can determine the genetic relatedness of the swine STEC strains but cannot provide information regarding gene content (74), future studies using sequence-based molecular methods, including whole-genome sequencing, can provide insight into the genetic diversity of swine STEC strains within and across farms. Overall, these results demonstrate a high level of diversity in virulence gene contents among the members of STEC populations from a small population of swine in the same geographic location and enhance our understanding of the transmission dynamics of STEC among pigs in the same finishing barn. However, whether swine STEC strains are potentially pathogenic to humans and the role swine play in the transmission of STEC to humans require further study.

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REFERENCES

1. **Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM.** 2011. Foodborne illness acquired in the United States—major pathogens. Emerg. Infect. Dis. **17:**7–15. http://dx.doi.org/10.3201/eid1701.P11101.
2. **Hoffmann S, Batz MB, Morris JG.** 2012. Annual cost of illness and quality-adjusted life year losses in the United States due to 14 foodborne pathogens. J. Food Prot. **75:**1292–1302. http://dx.doi.org/10.4315/0362-028X.JFP-11-417.
3. **Unkmeir A, Schmidt H.** 2000. Structural analysis of phage-borne *stx* genes and their flanking sequences in Shiga toxin-producing *Escherichia coli* and *Shigella dysenteriae* type 1 strains. Infect. Immun. **68:**4856–4864. http://dx.doi.org/10.1128/IAI.68.9.4856-4864.2000.
4. **O'Brien AD, Newland JW, Miller SF, Holmes RK, Smith HW, Formal SB.** 1984. Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. Science **226:**694–696. http://dx.doi.org/10.1126/science.6387911.
5. **McDaniel TK, Jarvis KG, Donnenberg MS, Kaper JB.** 1995. A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. Proc. Natl. Acad. Sci. U. S. A. **92:**1664–1668. http://dx.doi.org/10.1073/pnas.92.5.1664.
6. **Nataro JP, Kaper JB.** 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. **11:**142–201.
7. **Newton HJ, Sloan J, Bulach DM, Seemann T, Allison CC, Tauschek M, Robins-Browne RM, Paton JC, Whittam TS, Paton AW, Hartland EL.** 2009. Shiga toxin-producing *Escherichia coli* strains negative for locus of enterocyte effacement. Emerg. Infect. Dis. **15:**372–380. http://dx.doi.org/10.3201/eid1503.080631.
8. **Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL.** 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. Emerg. Infect. Dis. **11:**603–609. http://dx.doi.org/10.3201/eid1104.040739.
9. **Gyles CL.** 2007. Shiga toxin-producing *Escherichia coli*: an overview. J. Anim. Sci. **85:**E45–E62. http://dx.doi.org/10.2527/jas.2006-508.
10. **Paton AW, Ratcliff RM, Doyle RM, Seymour-Murray J, Davos D, Lanser JA, Paton JC.** 1996. Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga-like toxin-producing *Escherichia coli*. J. Clin. Microbiol. **34:**1622–1627.
11. **CDC.** 1995. Community outbreak of hemolytic uremic syndrome attributable to *Escherichia coli* O111:NM—South Australia 1995. MMWR Morb. Mortal. Wkly. Rep. **44:**550–558.
12. **CDC.** 1995. *Escherichia coli* O157:H7 outbreak linked to commercially distributed dry-cured salami—Washington and California, 1994. MMWR Morb. Mortal. Wkly. Rep. **44:**157–160.
13. **Williams RC, Isaacs S, Decou ML, Richardson EA, Buffett MC, Slinger RW, Brodsky MH, Ciebin BW, Ellis A, Hockin J.** 2000. Illness outbreak associated with *Escherichia coli* O157:H7 in Genoa salami. Can. Med. Assoc. J. **162:**1409–1413.
14. **MacDonald DM, Fyfe M, Paccagnella A, Trinidad A, Louie K, Patrick D.** 2004. *Escherichia coli* O157:H7 outbreak linked to salami, British Columbia, Canada, 1999. Epidemiol. Infect. **132:**283–289. http://dx.doi.org/10.1017/S0950268803001651.
15. **Conedera G, Mattiazzi E, Russo F, Chiesa E, Scorzato I, Grandesso S, Bessegato A, Fioravanti A, Caprioli A.** 2007. A family outbreak of *Escherichia coli* O157 haemorrhagic colitis caused by pork meat salami. Epidemiol. Infect. **135:**311–314. http://dx.doi.org/10.1017/S0950268806006807.
16. **Trotz-Williams LA, Mercer NJ, Walters JM, Maki AM, Johnson RP.** 2012. Pork implicated in a Shiga toxin-producing *Escherichia coli* O157:H7 outbreak in Ontario, Canada. Can. J. Public Health **103:**e322–e326.
17. **Fairbrother JM, Gyles CL.** 2012. Colibacillosis, p 723–747. *In* Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW (ed), Diseases of swine, 10th ed. John Wiley and Sons, West Sussex, United Kingdom.
18. **Fratamico PM, Bagi LK, Bush EJ, Solow BT.** 2004. Prevalence and characterization of Shiga toxin-producing *Escherichia coli* in swine feces recovered in the National Animal Health Monitoring System's Swine 2000 study. Appl. Environ. Microbiol. **70:**7173–7178. http://dx.doi.org/10.1128/AEM.70.12.7173-7178.2004.
19. **Borie C, Monreal Z, Guerrero P, Sanchez ML, Martinez J, Arellano C, Prado V.** 1997. Prevalence and characterization of enterohaemorrhagic *Escherichia coli* isolated from healthy cattle and pigs slaughtered in Santiago, Chile. Arch. Med. Vet. **29:**205–212. http://dx.doi.org/10.4067/S0301-732X1997000200005.
20. **Oporto B, Esteban JI, Aduriz G, Juste RA, Hurtado A.** 2008. *Escherichia coli* O157:H7 and non-O157 Shiga toxin-producing *E. coli* in healthy cattle, sheep and swine herds in northern Spain. Zoonoses Public Health **55:**73–81. http://dx.doi.org/10.1111/j.1863-2378.2007.01080.x.
21. **Karmali MA, Petric M, Lim C, Fleming PC, Steele BT.** 1983. *Escherichia coli* cytotoxin, haemolytic-uraemic syndrome, and haemorrhagic colitis. Lancet **ii:**1299–1300.
22. **Kaper JB, Nataro JP, Mobley HL.** 2004. Pathogenic *Escherichia coli*. Nat. Rev. Microbiol. **2:**123–140. http://dx.doi.org/10.1038/nrmicro818.
23. **Croxen MA, Finlay BB.** 2010. Molecular mechanisms of *Escherichia coli* pathogenicity. Nat. Rev. Microbiol. **8:**26–38. http://dx.doi.org/10.1038/nrmicro2265.
24. **Melton-Celsa A, Mohawk K, Teel L, O'Brien A.** 2012. Pathogenesis of Shiga-toxin producing *Escherichia coli*. Curr. Top. Microbiol. Immunol. **357:**67–103. http://dx.doi.org/10.1007/82_2011_176.
25. **Petruzziello-Pellegrini TN, Moslemi-Naeini M, Marsden PA.** 2013. New insights into Shiga toxin-mediated endothelial dysfunction in hemolytic uremic syndrome. Virulence **4:**556–563. http://dx.doi.org/10.4161/viru.26143.
26. **Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB.** 2013. Recent advances in understanding enteric pathogenic *Escherichia coli*. Clin. Microbiol. Rev. **26:**822–880. http://dx.doi.org/10.1128/CMR.00022-13.
27. **Endo Y, Tsurugi K, Yutsudo T, Takeda Y, Ogasawara T, Igarashi K.** 1988. Site of action of a Vero toxin (VT2) from *Escherichia coli* O157:H7 and of Shiga toxin on eukaryotic ribosomes. RNA N-glycosidase activity of the toxins. Eur. J. Biochem. **171:**45–50.
28. **Johannes L, Romer W.** 2010. Shiga toxins—from cell biology to biomedical applications. Nat. Rev. Microbiol. **8:**105–116. http://dx.doi.org/10.1038/nrmicro2279.
29. **Bugarel M, Martin A, Fach P, Beutin L.** 2011. Virulence gene profiling of enterohemorrhagic (EHEC) and enteropathogenic (EPEC) *Escherichia coli* strains: a basis for molecular risk assessment of typical and atypical EPEC strains. BMC Microbiol. **11:**142. http://dx.doi.org/10.1186/1471-2180-11-142.
30. **Frankel G, Phillips AD.** 2008. Attaching effacing *Escherichia coli* and paradigms of Tir-triggered actin polymerization: getting off the pedestal. Cell. Microbiol. **10:**549–556. http://dx.doi.org/10.1111/j.1462-5822.2007.01103.x.
31. **Paton AW, Srimanote P, Woodrow MC, Paton JC.** 2001. Characterization of Saa, a novel autoagglutinating adhesin produced by locus of enterocyte effacement-negative Shiga-toxigenic *Escherichia coli* strains that are virulent for humans. Infect. Immun. **69:**6999–7009. http://dx.doi.org/10.1128/IAI.69.11.6999-7009.2001.
32. **Low AS, Holden N, Rosser T, Roe AJ, Constantinidou C, Hobman JL, Smith DG, Low JC, Gally DL.** 2006. Analysis of fimbrial gene clusters and their expression in enterohaemorrhagic *Escherichia coli* O157:H7. Environ. Microbiol. **8:**1033–1047. http://dx.doi.org/10.1111/j.1462-2920.2006.00995.x.
33. **Galli L, Miliwebsky E, Irino K, Leotta G, Rivas M.** 2010. Virulence profile comparison between LEE-negative Shiga toxin-producing *Escherichia coli* (STEC) strains isolated from cattle and humans. Vet. Microbiol. **143:**307–313. http://dx.doi.org/10.1016/j.vetmic.2009.11.028.
34. **Brunder W, Schmidt H, Karch H.** 1996. KatP, a novel catalase-peroxidase encoded by the large plasmid of enterohaemorrhagic *Escherichia coli* O157:H7. Microbiology **142:**3305–3315. http://dx.doi.org/10.1099/13500872-142-11-3305.
35. **Coombes BK, Wickham ME, Mascarenhas M, Gruenheid S, Finlay BB, Karmali MA.** 2008. Molecular analysis as an aid to assess the public health risk of non-O157 Shiga toxin-producing *Escherichia coli* strains. Appl. Environ. Microbiol. **74:**2153–2160. http://dx.doi.org/10.1128/AEM.02566-07.
36. **Bolton DJ.** 2011. Verocytotoxigenic (Shiga toxin-producing) *Esche-*

richia coli: virulence factors and pathogenicity in the farm to fork paradigm. Foodborne Pathog. Dis. **8:**357–365. http://dx.doi.org/10.1089/fpd.2010.0699.
37. **Slanec T, Fruth A, Creuzburg K, Schmidt H.** 2009. Molecular analysis of virulence profiles and Shiga toxin genes in food-borne Shiga toxin-producing *Escherichia coli*. Appl. Environ. Microbiol. **75:**6187–6197. http://dx.doi.org/10.1128/AEM.00874-09.
38. **Fratamico PM, Bhagwat AA, Injaian L, Fedorka-Cray PJ.** 2008. Characterization of Shiga toxin-producing *Escherichia coli* strains isolated from swine feces. Foodborne Pathog. Dis. **5:**827–838. http://dx.doi.org/10.1089/fpd.2008.0147.
39. **Meng Q, Bai S, Zhao A, Lan R, Du H, Wang T, Shi C, Yuan S, Ji S, Jin D, Yu B, Wang Y, Sun H, Liu K, Xu J, Xiong Y.** 2014. Characterization of Shiga toxin-producing *Escherichia coli* isolated from healthy pigs in China. BMC Microbiol. **14:**5. http://dx.doi.org/10.1186/1471-2180-14-5.
40. **Chapman TA, Wu XY, Barchia I, Bettelheim KA, Driesen S, Trott D, Wilson M, Chin JJ.** 2006. Comparison of virulence gene profiles of *Escherichia coli* strains isolated from healthy and diarrheic swine. Appl. Environ. Microbiol. **72:**4782–4795. http://dx.doi.org/10.1128/AEM.02885-05.
41. **Zweifel C, Schumacher S, Beutin L, Blanco J, Stephan R.** 2006. Virulence profiles of Shiga toxin 2e-producing *Escherichia coli* isolated from healthy pig at slaughter. Vet. Microbiol. **117:**328–332. http://dx.doi.org/10.1016/j.vetmic.2006.06.017.
42. **Sonntag AK, Bielaszewska M, Mellmann A, Dierksen N, Schierack P, Wieler LH, Schmidt MA, Karch H.** 2005. Shiga toxin 2e-producing *Escherichia coli* isolates from humans and pigs differ in their virulence profiles and interactions with intestinal epithelial cells. Appl. Environ. Microbiol. **71:**8855–8863. http://dx.doi.org/10.1128/AEM.71.12.8855-8863.2005.
43. **Tseng M, Fratamico PM, Bagi L, Manzinger D, Funk JA.** 8 May 2014. Shiga toxin-producing *E. coli* (STEC) in swine: prevalence over the finishing period and characteristics of the STEC isolates. Epidemiol. Infect. http://dx.doi.org/10.1017/S0950268814001095.
44. **Bugarel M, Beutin L, Martin A, Gill A, Fach P.** 2010. Micro-array for the identification of Shiga toxin-producing *Escherichia coli* (STEC) seropathotypes associated with hemorrhagic colitis and hemolytic uremic syndrome in humans. Int. J. Food Microbiol. **142:**318–329. http://dx.doi.org/10.1016/j.ijfoodmicro.2010.07.010.
45. **Delannoy S, Beutin L, Fach P.** 2013. Discrimination of enterohemorrhagic *Escherichia coli* (EHEC) from non-EHEC strains based on detection of various combinations of type III effector genes. J. Clin. Microbiol. **51:**3257–3262. http://dx.doi.org/10.1128/JCM.01471-13.
46. **Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, Barrett TJ.** 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne Pathog. Dis. **3:**59–67. http://dx.doi.org/10.1089/fpd.2006.3.59.
47. **Tarr PI, Bilge SS, Vary JC, Jelacic S, Jr, Habeeb RL, Ward TR, Baylor MR, Besser TE.** 2000. Iha: a novel *Escherichia coli* O157:H7 adherence-conferring molecule encoded on a recently acquired chromosomal island of conserved structure. Infect. Immun. **68:**1400–1407. http://dx.doi.org/10.1128/IAI.68.3.1400-1407.2000.
48. **Doughty S, Sloan J, Bennett-Wood V, Robertson M, Robins-Browne RM, Hartland EL.** 2002. Identification of a novel fimbrial gene cluster related to long polar fimbriae in locus of enterocyte effacement-negative strains of enterohemorrhagic *Escherichia coli*. Infect. Immun. **70:**6761–6769. http://dx.doi.org/10.1128/IAI.70.12.6761-6769.2002.
49. **Frydendahl K.** 2002. Prevalence of serogroups and virulence genes in *Escherichia coli* associated with postweaning diarrhoea and edema disease in pigs and a comparison of diagnostic approaches. Vet. Microbiol. **85:**169–182. http://dx.doi.org/10.1016/S0378-1135(01)00504-1.
50. **Benz I, Schmidt MA.** 1992. Isolation and serologic characterization of AIDA-I, the adhesin mediating the diffuse adherence phenotype of the diarrhea-associated *Escherichia coli* strain 2787 (O126:H27). Infect. Immun. **60:**13–18.
51. **Benz I, Schmidt MA.** 1989. Cloning and expression of an adhesin (AIDA-I) involved in diffuse adherence of enteropathogenic *Escherichia coli*. Infect. Immun. **57:**1506–1511.
52. **An H, Fairbrother JM, Desautels C, Harel J.** 1999. Distribution of a novel locus called Paa (porcine attaching and effacing associated) among enteric *Escherichia coli*. Adv. Exp. Med. Biol. **473:**179–184. http://dx.doi.org/10.1007/978-1-4615-4143-1_17.
53. **Savarino SJ, Fasano A, Robertson DC, Levine MM.** 1991. Enteroaggregative *Escherichia coli* elaborate a heat-stable enterotoxin demonstrable in an in vitro rabbit intestinal model. J. Clin. Invest. **87:**1450–1455. http://dx.doi.org/10.1172/JCI115151.
54. **Dallas WS.** 1990. The heat-stable toxin I gene from *Escherichia coli* 18D. J. Bacteriol. **172:**5490–5493.
55. **Welch RA, Forestier C, Lobo A, Pellett S, Thomas W, Jr, Rowe G.** 1992. The synthesis and function of the *Escherichia coli* hemolysin and related RTX exotoxins. FEMS Microbiol. Immunol. **5:**29–36.
56. **Abu-Ali GS, Lacher DW, Wick LM, Qi W, Whittam TS.** 2009. Genomic diversity of pathogenic *Escherichia coli* of the EHEC 2 clonal complex. BMC Genomics **10:**296. http://dx.doi.org/10.1186/1471-2164-10-296.
57. **Brandt SM, King N, Cornelius AJ, Premaratne A, Besser TE, On SL.** 2011. Molecular risk assessment and epidemiological typing of Shiga toxin-producing *Escherichia coli* by using a novel PCR binary typing system. Appl. Environ. Microbiol. **77:**2458–2470. http://dx.doi.org/10.1128/AEM.02322-10.
58. **Kaufmann M, Zweifel C, Blanco M, Blanco JE, Blanco J, Beutin L, Stephan R.** 2006. *Escherichia coli* O157 and non-O157 Shiga toxin-producing *Escherichia coli* in fecal samples of finished pigs at slaughter in Switzerland. J. Food Prot. **69:**260–266.
59. **Ateba CN, Mbewe M.** 2011. Detection of *Escherichia coli* O157:H7 virulence genes in isolates from beef, pork, water, human and animal species in the northwest province, South Africa: public health implications. Res. Microbiol. **162:**240–248. http://dx.doi.org/10.1016/j.resmic.2010.11.008.
60. **Lenahan M, O'Brien SB, Byrne C, Ryan M, Kennedy CA, McNamara EB, Fanning S, Sheridan JJ, Sweeney T.** 2009. Molecular characterization of Irish *E. coli* O157:H7 isolates of human, bovine, ovine and porcine origin. J. Appl. Microbiol. **107:**1340–1349. http://dx.doi.org/10.1111/j.1365-2672.2009.04320.x.
61. **Hauser E, Mellmann A, Semmler T, Stoeber H, Wieler LH, Karch H, Kuebler N, Fruth A, Harmsen D, Weniger T, Tietze E, Schmidt H.** 2013. Phylogenetic and molecular analysis of food-borne Shiga toxin-producing *Escherichia coli*. Appl. Environ. Microbiol. **79:**2731–2740. http://dx.doi.org/10.1128/AEM.03552-12.
62. **Friedrich AW, Bielaxzewska M, Zhang WL, Pulz M, Kuczius T, Ammon A, Karch H.** 2002. *Escherichia coli* harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. J. Infect. Dis. **185:**74–84. http://dx.doi.org/10.1086/338115.
63. **Beutin L, Krause G, Zimmermann S, Kaulfuss S, Gleier K.** 2004. Characterization of Shiga toxin-producing *Escherichia coli* strains isolated from human patients in Germany over a 3-year period. J. Clin. Microbiol. **42:**1099–1108. http://dx.doi.org/10.1128/JCM.42.3.1099-1108.2004.
64. **Orth D, Grif K, Khan AB, Naim A, Dierich MP, Wurzner R.** 2007. The Shiga toxin genotype rather than the amount of Shiga toxin or the cytotoxicity of Shiga toxin in vitro correlates with the appearance of the hemolytic uremic syndrome. Diagn. Microbiol. Infect. Dis. **59:**235–242. http://dx.doi.org/10.1016/j.diagmicrobio.2007.04.013.
65. **Bielaszewska M, Friedrich AW, Aldick T, Schurk-Bulgrin R, Karch H.** 2006. Shiga toxin activatable by intestinal mucus in *Escherichia coli* isolated from humans: predictor for a severe clinical outcome. Clin. Infect. Dis. **43:**1160–1167. http://dx.doi.org/10.1086/508195.
66. **Thomas A, Cheasty T, Chart H, Rowe B.** 1994. Isolation of Vero cytotoxin-producing *Escherichia coli* serotypes O9ab:H- and O101:H-carrying VT2 variant gene sequences from a patient with haemolytic uraemic syndrome. Eur. J. Clin. Microbiol. Infect. Dis. **13:**1074–1076. http://dx.doi.org/10.1007/BF02111832.
67. **Pierard D, Huyghens L, Lauwers S, Lior H.** 1991. Diarrhoea associated with *Escherichia coli* producing porcine oedema disease verotoxin. Lancet **338:**762.
68. **Muniesa M, Recktenwald J, Bielaszewska M, Karch H, Schmidt H.** 2000. Characterization of a Shiga toxin 2e-converting bacteriophage from an *Escherichia coli* strain of human origin. Infect. Immun. **68:**4850–4855. http://dx.doi.org/10.1128/IAI.68.9.4850-4855.2000.
69. **Johnsen G, Wasteson Y, Heir E, Berget OI, Herikstad H.** 2001. *Escherichia coli* O157:H7 in faeces from cattle, sheep and pigs in the southwest part of Norway during 1998 and 1999. Int. J. Food Microbiol. **65:**193–200. http://dx.doi.org/10.1016/S0168-1605(00)00518-3.
70. **Franke S, Harmsen D, Caprioli A, Pierard D, Wieler LH, Karch H.** 1995. Clonal relatedness of Shiga-like toxin-producing *Escherichia coli* O101 strains of human and porcine origin. J. Clin. Microbiol. **33:**3174–3178.

71. **Osek J, Gallien P.** 2002. Molecular analysis of *Escherichia coli* O157 strains isolated from cattle and pigs by the use of PCR and pulsed-field gel electrophoresis methods. Vet. Med. (Praha) **47:**149–158.
72. **Rios M, Prado V, Trucksis M, Arellano C, Borie C, Alexandre M, Fica A, Levine MM.** 1999. Clonal diversity of Chilean isolates of enterohemorrhagic *Escherichia coli* from patients with hemolytic-uremic syndrome, asymptomatic subjects, animal reservoirs, and food products. J. Clin. Microbiol. **37:**778–781.
73. **Joris MA, Verstraete K, De Reu K, De Zutter L.** 2013. Longitudinal follow-up of the persistence and dissemination of EHEC on cattle farms in Belgium. Foodborne Pathog. Dis. **10:**295–301. http://dx.doi.org/10.1089/fpd.2012.1277.
74. **Singer RS, Sischo WM, Carpenter TE.** 2004. Exploration of biases that affect the interpretation of restriction fragment patterns produced by pulsed-field gel electrophoresis. J. Clin. Microbiol. **42:**5502–5511. http://dx.doi.org/10.1128/JCM.42.12.5502-5511.2004.