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Prevalence and Characterization of Virulence Genes in *Escherichia coli* Isolated from Diarrheic Piglets in Korea

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ABSTRACT - Enterotoxigenic *Escherichia coli* is one of the major causative infectious agents of diarrhea in newborn and post-weaning pigs and leads to a large economic loss worldwide. However, there is limited information on the distribution and characterization of virulence genes in *E. coli* isolated from diarrheic piglets, which also applies to the current status of pig farms in Korea. To investigate the prevalence and characterization of virulence genes in *E. coli* related to diarrhea in piglets, the rectal swab samples of diarrheic piglets (aged 2 d to 6 w) were collected from 163 farms between 2013 and 2016. Five to 10 individual swab samples from the same farm were pooled and cultured on MacConkey agar plates, and *E. coli* were identified using the API 32E system. Three sets of multiplex PCRs were used to detect 13 *E. coli* virulence genes. As a result, a total of 172 *E. coli* isolates encoding one or more of the virulence genes were identified. Among them, the prevalence of individual virulence gene was as follows, (1) fimbrial adhesins (43.0%): F4 (16.9%), F5 (4.1%), F6 (1.7%), F18 (21.5%), and F41 (3.5%); (2) toxins (90.1%): LT (19.2%), STa (20.9%), STb (25.6%), Stx2e (15.1%), EAST1 (48.3%); and (3) non-fimbrial adhesin (19.6%): EAE (14.0%), AIDA-1 (11.6%) and PAA (8.7%), respectively. Taken together, various pathotypes and virotypes of *E. coli* were identified in diarrheic piglets. These results suggest a broad array of virulence genes is associated with coliform diarrhea in piglets in Korea.

Key words : Diarrheic piglet, *E. coli*: Prevalence, Virulence factor

Escherichia coli is an important causative agent of diarrhea in newborn and post-weaning pigs, causing significant losses in large-scale pig farms worldwide. Enterotoxigenic and shiga toxin-producing *E. coli* (ETEC and STEC) are the main categories of pathogenic *E. coli* that cause infectious diseases in pigs[1,2].

The virulence characteristics of ETEC are strongly dependent on the production of fimbrial adhesins and enterotoxins. The adherence of bacteria mediated by fimbrial adhesins and production of enterotoxins are necessary for the occurrence of disease. The bacteria attach to and colonize the intestinal lining, and then secrete the enterotoxins[2,3]. The porcine ETEC isolates produce one or more major fimbrial adhesins, including F4 (K88), F5 (K99), F6 (987P), F18, and F41[3-5]. The enterotoxins produced by ETEC include the heat-labile enterotoxin (LT), heat-stable enterotoxin type A (STa), and heat-stable enterotoxin type B (STb)[2,6,7]. Another heat-stable toxin, enteroaggregative *E. coli* heat-stable enterotoxin 1 (EAST1) is highly prevalent in the ETEC strains isolated from human and animals with diarrhea. However, its role of the development of diarrhea has not been elucidated[8-11]. The shiga toxin 2e (Stx2e) produced by STEC is associated with diarrhea, bloody stool, sudden death, and edema disease in pigs[12,13]. Shiga toxin-producing *E. coli* is mainly isolated from pigs with edema disease. However, there are also many reports showing the production of shiga toxin by the *E. coli* isolates from diarrheic pigs, suggesting its role in the pathogenesis of post weaning diarrhea (PWD)[12-15].

Three virulence factors have recently been reported to be associated with porcine diarrhea. Adhesin involved in diffuse adherence (AIDA-I), an outer membrane protein produced by di-usely adherent *E. coli* (DAEC), induces diffuse adherence (DA) pattern lesion on the intestinal mucosa[16-18]. The AIDA-1 positive *E. coli* induced diarrhea

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in the colostrum-deprived neonatal piglets, and AIDA-1 played an essential role in the adhesion to the intestinal epithelial cell[17,18]. Further, AIDA-1 was first identified in the *E. coli* strain 2787 isolated from a human infantile diarrhea case[16]. Since then, the AIDA-1 positive *E. coli* strains have been found in both human and pigs[11,18-20]. The prevalence of AIDA-1 positive *E. coli* in pigs is relatively high compared with that in human, and the porcine *E. coli* strains are considered to be a major reservoir of the *AIDA-1* genes[11,18-21].

The porcine attaching and effacing-associated (pAA) and *E. coli* attaching and effacing (EAE) factors have been frequently found in the *E. coli* isolates from diarrheic pigs[8,11,22,23]. Furthermore, pAA and EAE are outer membrane proteins (intimin) produced by enteropathogenic *E. coli* (EPEC), and the gene encoding these factors is located in locus of enterocyte effacement (LEE)[22,24,25]. These two factors are associated with porcine diarrhea by adhering to the intestinal epithelial cells with a characteristic attaching and effacing (A/E) pattern lesion[24,26,27]. Furthermore, pAA is found in porcine enteropathogenic *E. coli* (PEPEC), and its pathogenicity is limited to pigs[22,24], whereas EAE is usually found in EPEC in both human and other animals. Enteropathogenic *E. coli* is a leading causative agent of infantile diarrhea in developing countries, and can cause porcine diarrhea[25,27]. This pathotype is subdivided into typical and atypical EPEC according to their virulence markers. The typical strains have EPEC adherence factor (EAF) plasmid and the gene for bundle-forming pili (BFP). Whereas, the atypical strains do not have these factors and are therefore less virulent[25]. Most EPEC strains isolated from pigs are atypical EPEC[28], and the atypical EPEC strains are predominant in industrialized countries[25].

In the present study, we (1) examined the above mentioned 13 virulence genes among the *E. coli* strains recently isolated from diarrheic piglets in Korea through the polymerase chain reaction and (2) investigated the prevalence and distribution of these virulence genes. The results of the present study will provide information, regarding the distribution of different virulence factors in *E. coli* causing diarrhea in pigs, which is required to control diarrheic disease in piglets due to pathogenic *E. coli*.

Materials and Methods

Sample collection and *E. coli* isolation

Between 2013 and 2016, the rectal swab samples of piglets (aged 2 d to 6 w) with diarrhea were collected from 163 farms in South Korea. Five to 10 samples were collected during diarrhea outbreak in each farm. The samples were submitted to the Research and Development Unit of the Green Cross Veterinary Products (Young-in, Korea), where they were pooled and cultured directly on eosin methylene blue (EMB) agar (BD-Difco, Franklin Lakes, NJ, USA). Subsequently, metallic green sheen colonies on EMB agar were selected, and then cultured on MacConkey agar (BD-Difco). Subsequently, red/pink colonies on MacConkey agar were selected, and were confirmed as *E. coli* using the API 32E system (BioMerieux, Marcy l'Etoile, France)[29]. The identified *E. coli* isolates were stored at -70ºC until used.

Polymerase chain reaction (PCR) to identify the virulence factors

The primers to amplify the genes encoding fimbria (F4,

Table 1. Polymerase chain reaction primers for the virulence genes of *E. coli* isolates

Virulence gene		Forward (5′-3′)	Reverse (3′-5′)	Product size (bp)
Set1	*F4* (K88)	TGAATGACCTGACCAATGGTGGAACC	GCGTTTACTCTTTGAATCTGTCCGAG	484
	F5 (K99)	GCGACTACCAATGCTTCTGCGAATAC	GAACCAGACCAGTCAATACGAGCA	230
	F18	TGGCACTGTAGGAGATACCATTCAG	GGTTTGACCACCTTTCAGTTGAGCAG	334
	Stx2e	CGGTATCCTATTCCCAGGAGTTTACG	GTCTTCCGGCGTCATCGTATAAACAG	599
	STb	GCTACAAATGCCTATGCATCTACACA	CATGCTCCAGCAGTACCATCTCTAAC	125
Set2	*F6* (987P)	GCCAGTCTATGCCAAGTGGATACTTC	GTTTGTATCAGGATTCCCTGTGGTGG	391
	F41	TTAGCAGCGAAGATGAGTGATGGG	GTACTACCTGCAGAAACACCAGATCC	515
	LT	ACGGCGTTACTATCCTGTCTATGTGC	TTGGTCTCGGTCAGATATGTGATTCT	275
	STa	GTCAGTCAACTGAATCACTTGACTCT	CATGGAGCACAGGCAGGATTACAACA	152
Set3	*AIDA-1*	ACAGTATCATATGGAGCCA	TGTGCGCCAGAACTATTA	585
	EAE	CATTATGGAACGGCAGAGGT	ATCTTCTGCGTACTGCGTTCA	790
	PAA	CCATAAAGACAGCTTCAGTGAAAA	GTATTACTGGTACCACCACCATCA	180
	EAST1	TCGGATGCCATCAACACAGT	GTCGCGAGTGACGGCTTTGTAG	125

F5, F6, F18, and F41), toxins (LT, STa, STb, Stx2e, and EAST1), and pAA have been described by Zhang et al[11]. The primers to amplify the genes encoding AIDA-1 and EAE have been described by Benz and Schmidt and Beaudry et al., respectively[30,31]. The primer pairs for the 13 virulence factors were used for three sets of multiplex PCR (Table 1). The PCR was performed according to the protocol described by Zhang et al.[11] using BioMetra TOne PCR machine (Analytik Jena AG, Jena, Germany). The reference *E. coli* strains used were E-174 (F4+, F6+, LT+, STa+, STb+, and pAA+) and E-92 (F5+, F41+, and STa+), which were supplied by the Animal and Plant Quarantine agency (Gim-cheon, Korea). Additionally, the *E. coli* strains S85 (F18+ and STx2e+) and G-547 (AIDA-1+, EAE+, and EAST1+) isolated in the present study were used as references.

Characterization and classification of *E. coli* isolates

When the *E. coli* isolates with the same combination of virulence factors (virotype) were detected in the same case, they were considered duplicates of an *E. coli* prototype strain. Only the prototype (not the duplicates) was considered to calculate prevalence. The *E. coli* isolates were classified into different pathotypes according to the genes encoding virulence factors (each representing a pathotype), as previously proposed[29]. Briefly, the isolates with the gene for fimbrial adhesion (F4, F5, F6, F41, and F18) and enterotoxin (STa, STb, and LT) were classified into ETEC. The isolates with the gene encoding shiga-toxin (Stx2e) and AIDA-1 were classified into STEC and DAEC, respectively. The isolates with the gene encoding EAE and pAA were classified into EPEC. In addition, EAST1 was considered as the virulence factor representing an independent pathotype.

Results

Prevalence of individual virulence genes

The *E. coli* isolates were recovered from the rectal swab samples of diarrheic piglets in 163 farms. A total of 172 isolates with one or more of the virulence genes were identified in 121 farms (74.2%). Among them, 104 and 68 *E. coli* isolates were recovered from pre-weaning and post-weaning piglets, respectively, and there were no significant differences in the prevalence of individual virulence genes between them (Table 2).

Among the 172 isolates, the genes encoding fimbrial adhesins were identified in 74 isolates (43.0%). The number of isolates with genes encoding different fimbrial adhesins was as follows: 29 (16.9%), 7 (4.1%), 3 (1.7%), 37 (21.5%), and 6 (3.5%) for F4, F5, F6, F18, and F41, respectively. Most of these isolates carried a single fimbrial adhesin-encoding gene and only eight isolates carried two fimbrial adhesin-encoding genes. The most common fimbrial adhesin-encoding genes were *F18* (43.2%), followed by *F4* (33.8%), in the 74 isolates. Most of the isolates investigated carried the toxin-encoding genes. Among the 172 isolates, 155 isolates (90.1%) carried at least one toxin-encoding gene, and the number of isolates with genes encoding different toxins was as follows: 33 (19.2%), 36 (20.9%), 44 (25.6%), 27 (15.7%), and 83 (48.3%) for LT, STa, STb, Stx2e, and EAST1, respectively. The most common toxin-encoding genes were *EAST1* and *STb*. The prevalence of both LT and STa was similar. Among the 172 isolates, 49 isolates (28.5%) carried the non-fimbrial adhesin-encoding genes. The most common non-fimbrial adhesin-encoding gene was *EAE* (14.0%, 24 out of 172), followed by *AIDA-1* (11.6%, 20 out of 172) and *pAA* (8.7%, 15 out of 172) (Table 2).

Identification of pathotypes and virotypes

Among the 172 isolates, 99 (57.6%), 28 (16.3%), 34 (19.8%), 20 (11.6%), and 83 (58.4%) isolates carried the genes for ETEC, STEC, EPEC, DAEC, and EAST1, respectively. Among the 99 ETEC, 28 STEC, 34 EPEC, 20 DAEC, and 83 EAST1 isolates, 38 (38.4%), 4 (14.3%), 15 (44.1%), 1 (5%), and 41 (49.4%) isolates carried the genes

Table 2. Prevalence of the virulence genes in the 172 *Escherichia coli* isolates from pre-weaning and post-weaning piglets

	Fimbrial adhesin					Toxin				Non-fimbrial adhesin			
	F4	*F5*	*F6*	*F18*	*F41*	*LT*	*STa*	*STb*	*Stx2e*	*EAST1*	*AIDA-1*	*EAE*	*PAA*
Pre-weaning (104)[a]	16.3%[b] (17)	2.9% (3)	1.0% (1)	20.2% (21)	2.9% (3)	18.3% (19)	19.2% (20)	25.0% (26)	14.4% (15)	49.0% (51)	10.6% (11)	12.5% (13)	7.7% (8)
Post-weaning (68)	17.6% (12)	5.9% (4)	2.9% (2)	23.5% (16)	4.4% (3)	22.1% (15)	23.5% (16)	26.5% (18)	17.6% (12)	47.1% (32)	13.2% (9)	16.2% (11)	10.3% (7)
Total (172)	16.9% (29)	4.1% (7)	1.7% (3)	21.5% (37)	3.5% (6)	19.8% (34)	25.6% (36)	44% (25.6)	15.7% (27)	48.3% (83)	11.6% (20)	14.0% (24)	8.7% (15)

[a] The total number of isolates from diarrheic piglets encoding one or more of the virulence genes. [b]The percent of individual virulence gene at each stage (pre-weaning, post-weaning, and all ages).

for only ETEC, STEC, EPEC, DAEC, and EAST1, respectively (Table 2). The remaining 73 isolates (42.4%) carried the genes for more than two pathotype-representative factors, and can be classified into the following 14 pathotypes. Twenty (11.6%), 16 (9.3%), 7 (4.1%), 6 (3.5%), and 4 (2.3%) isolates carried the genes for ETEC/EAST1, ETEC/STEC, EPEC/EAST1, ETEC/DAEC/EAST1, and ETEC/EPEC/EAST1, respectively. The number of isolates with the following four pathotypes ETEC/DAEC, ETEC/EPEC, DAEC/EAST1, and EPEC/DAEC/EAST1 was 2 (1.2%) per pathotype. The number of isolates with the following five pathotypes EPEC/DAEC, ETEC/STEC/DAEC, ETEC/EPEC/DAEC, ETEC/EPEC/EAST1, and ETEC/EPEC/DAEC/ EAST1 was 1 (0.6%) per pathotype.

In the present study, 78 different virotypes were identified, which are presented in Table 3. There was no predominant virotype, except EAST1 positive isolates (16.5%, 41 out of 248). Further, 49 and 30 virotypes were identified from the fimbrial and non-fimbrial isolates, respectively. In the fimbrial isolates, the most diverse virotypes were observed in the F18 fimbrial isolates (23 virotypes), followed by the F4 fimbrial isolates (11 virotypes). In the F4 fimbrial

Table 3. Fimbrial adhesin, toxin, and non-fimbrial adhesin profiles of the *Escherichia coli* isolates from diarrheic piglets

Toxin profile	Fimbrial and non-fimbrial adhesins; no. of isolates carrying the genes												
	F4	*F5*	*F6*	*F41*	*F18*	*AIDA-1*	*PAA*	*EAE*	Fimbrial adhesins[a]	Non-fimbrial adhesins[b]	Fimbrial and Non-fimbrial adhesins[c]	None	Total
LT					1	1				1		1	4
STa	3	1			2	1		1				3	11
STb	1	2					1		2		1		7
EAST1			2		1	2	2	5		2		41	55
Stx2e				1	4						1	4	10
LT/STa	2				1				1				4
LT/STb	5				1						2	1	9
LT/Stx2e					1								1
LT/EAST1	3				1				1			1	6
STa/STb	1				1				1	1	1	1	6
STa/EAST1					1					1		1	3
STa/Stx2e					3							2	5
STb/EAST1	2					5			1	1		1	10
STb/Stx2e					2								2
EAST1/Stx2e					2								2
LT/STa/STb					1								1
LT/STa/EAST1					1								1
LT/STb/EAST1	3					1							4
LT/STa/Stx2e					1								1
LT/STb/Stx2e	1				1								2
STa/Stx2e/EAST1					1								1
STa/STb/EAST1/Stx2e												1	1
STa/STb/LT/Stx2e					1								1
None	2			1	2	1	3	9	1	4	2		25
Total	23	3	2	2	29	11	6	15	7	10	7	57	172

[a]Isolates carrying two genes for fimbrial adhesins; K88/K99/STb (n=1), K88/987p/LT/EAST1 (n=1), K88/F18/STb (n=1), K88/F18/STa/STb (n=1), K99/F18/LT/STa (n=1), K99/F41/STb/EAST1 (n=1), and F18/F41(n=1).

[b]Isolates carrying two genes for non-fimbrial adhesins; AIDA-1/PAA (n=1), AIDA-1/EAE/LT (n=1), AIDA-1/EAE/EAST1 (n=2), PAA/EAE (n=3), EAE/PAA/STb/EAST1 (n=1), AIDA-1/EAE /STa/EAST1 (n=1), and EAE/PAA/STa/STb (n=1).

[c]Isolates carrying the genes for both fimbrial and non-fimbrial adhesins; K88/PAA/LT/STb (n=2), K99/AIDA-1/STb (n=1), F18/AIDA-1/Stx2e (n=1), F18/PAA/STa/STb (n=1), F41/AIDA-1 (n=1), and F18/F41/AIDA-1 (n=1).

isolates, F4/LT/STb (21.7%, 5 out of 23) was the most prevalent, followed by F4/STa (13.0%, 3 out of 23), F4/LT/EAST1 (13.0%, 3 out of 23), and F4/LT/STb/Stx2e (13.0%, 3 out of 23). In the F18 fimbrial isolates, F18/Stx2e (16.7%, 5 out of 30) was the most prevalent, followed by F18/STa/Stx2e (10.0%, 3 out of 30).

Distribution of toxin and non-fimbrial genes in the fimbrial isolates

Among the 172 isolates, except EAST1, most of investigated toxins strongly associated with the fimbrial adhesins. Among the 34, 36, 44, and 27 isolates with the genes encoding LT, STa, STb, and Stx2e, 30 (90.9%), 26 (72.2%), 35 (79.5%), and 19 (73.1%) isolates carried the genes for fimbrial adhesin, respectively (Table 2). Among the fimbrial adhesins, LT and STb more strongly associated with F4 than F18, respectively. However, STa and Stx2e more strongly associated with F18 than F4, respectively. Especially, the *Stx2e* gene was significantly associated with the *F18* gene. Among the 19 fimbrial isolates with the *Stx2e* gene, 17 (89.5%) isolates carried the *F18* gene. However, EAST1 and the non-fimbrial adhesins did not significantly correlate with the fimbrial adhesins. Among the 83, 20, and 15 isolates with the *EAST1*, *AIDA-1*, and *pAA* genes, respectively, 61 (73.5%), 15 (75.0%), and 12 (80%) isolates did not carry any gene encoding fimbrial adhesins. Especially, all the 24 isolates with the *EAE* gene did not carry any gene encoding fimbrial adhesins (Table 3).

Discussion

One hundred and seventy-two isolates with one or more of the virulence genes was recovered from diarrheic piglets in the present study. These isolates were classified into several pathotypes according to the virulence genes they possessed. Majority of the isolates belonged to the ETEC category, which is similar to the findings of previous studies[29,32].

It is known that F4 and F18 are the major fimbrial adhesins associated with ETEC isolated from diarrheic piglets[3-5,11,32]. Similarly, even in the present study F4 and F18 were the predominant fimbrial adhesins. Furthermore, there are some differences in terms of the most prevalent fimbrial adhesin depending on the country or continent. A higher prevalence of F4 than F18 has been reported in the USA and Europe[11,32]. Whereas, F18 is the most prevalent in eastern China and South Korea[8,29]. Similarly, in the present study, F18 exhibited the highest prevalence among the fimbrial adhesins.

STa, STb, and LT are the major enterotoxins produced by ETEC, and these toxins are mainly found with fimbrial adhesins in ETEC[3,7]. In the present study, 90.9%, 72.2%, and 79.5% of the fimbrial isolates carried the *LT*, *STa*, and *STb* genes, respectively. Several studies have indicated that STb is the most prevalent in the *E. coli* strains isolated from diarrheic piglets[8,10,11,32-35]. Similarly, in the present study, the most prevalent enterotoxin was STb, followed by STa and LT. In addition, the prevalence pattern of these toxins was similar to that reported in Europe[32], indicating that LT and STa are more widespread than STb[29]. The *STb* gene is considered to be widespread among the pathogenic *E. coli* strains causing porcine diarrhea in South Korea since 2010.

In the present study, the *LT* (56.7%, 17 out of 30) and *STb* (51.4%, 18 out of 35) genes were highly associated with the *F4* gene. Whereas, the *STa* gene was strongly associated with the *F18* gene (65.4%, 17 out of 26) when compared with that of the *F4* gene (26.9%, 7 out of 26). A similar association has been reported between *F4* and *LT* and between *F4* and *STb*, but not between *F4* and *STa* in the USA and Slovakia[11,36]. Interestingly, there was a significant increase in STa among the F4 fimbrial isolates encoding enterotoxins in Canada in 1998-2001 and 1974-1987[37]. However, the high frequency of F4 fimbrial isolates with the *STa* gene was not observed in the present study.

Although the pathogenic significance of EAST1 toxin has not been well explored[6,11], many studies have indicated a high prevalence of the *EAST1* gene in *E. coli* isolates from pigs with diarrhea and/or edema disease[8-11,29]. Furthermore, the high prevalence (48.3%, 83 out of 172) of *EAST1* was found in the present study. Choi et al.[9] and Kim et al.[29] reported that 44.3% and 52.0% of the EAST1-positive isolates carried *EAST1* as the only toxin gene. Similarly, in the present study 49.4% of the EAST1-positive isolates carried *EAST1* as the only toxin gene. This high frequency of isolates with *EAST1* as the only toxin gene suggests that this toxin alone is likely implicated in porcine diarrhea without synergic effect with other toxins.

Lee et al.[34] indicated a very low frequency (2.3%) of the *Stx2e* gene in the 171 isolates with one or more of the virulence genes from diarrheic piglets, whereas, Kim et al.[29] indicated relatively high frequency (15.2%) of *Stx2e* in the isolates with one or more of the virulence genes from diarrheic piglets in South Korea[29,34]. In the present study, the prevalence of the *Stx2e* gene was 26.0% (26 out of 172), suggesting an increasing trend in South Korea. These results indicate that Stx2e strongly affects porcine diarrhea in South Korea. Generally, it is known that the *Stx2e* gene is strongly associated with the *F18* gene among the fimbrial isolates of ETEC[29]. Similarly, the results of the present study supported the association between the *F18* and *Stx2e* genes. Furthermore, 89.5% of the fimbrial isolates with the *Stx2e* gene carried the *F18* gene. Currently, most of the

commercialized vaccines for swine colibacillosis do not contain Stx2e and F18 antigens. Considering the high prevalence of Stx2e and F18, it is necessary to take countermeasures against these virulence factors, such as development of new vaccine to control piglet diarrhea more effectively.

Previous studies have indicated an association between fimbrial adhesin and certain toxinotypes. In the USA and Europe, F4 and F18 strongly associated with LT/STb and STa/STb/STx2e, respectively[11,32]. Chen et al.[8] indicated that F4 and F18 strongly associated with STa only in eastern China. In the present study, F4/LT/STb was the most prevalent in the F4 fimbrial isolates, although it was not significantly predominant. In the F18 fimbrial isolates, although F18/Stx2e was the most prevalent, there was no predominant virotype.

Lee and his colleagues reported that 2.9% of the 171 *E. coli* isolates with one or more of the virulence genes in South Korea carried the *AIDA-1* gene[34]. In the present study, 11.6% of the 172 isolates carried the *AIDA-1* gene, and its prevalence was about four times higher than that reported by Lee and his colleagues. This increase in the *AIDA-1* gene suggests that this virulence factor is widespread among the pathogenic *E. coli* causing porcine diarrhea during the past decade in South Korea. Whereas, 8.7% of the 172 isolates carried the *pAA* gene, which is similar to the result (8.7% of the 171 isolates) of a previous study in South Korea[34]. Further, EAE showed a very low frequency of 1.5% (3 out of 198) and 2.5% (6 out of 238) in the *E. coli* isolates with one or more of the virulence genes from diarrheic piglets in Denmark[10] and the USA[11], respectively. Whereas, Kim et al.[29] indicated a very high frequency of the *EAE* gene with 20.9% (40 out of 191) in the *E. coli* isolates with one or more of the virulence genes from diarrheic piglets in South Korea. In the present study, the *EAE* gene exhibited higher prevalence (14.0%) than that reported in other countries. The pathogenicity of pAA is limited to pigs[22,24], whereas AIDA-1 and EAE can affect pigs and humans, and these factors have been found in pathogenic *E. coli* strains isolated from pigs and humans[17,19,25,28]. The high prevalence of the *AIDA-1* and *EAE* genes in *E. coli* isolated from pigs in the present study, suggests that the porcine *E. coli* strains appear to be a major reservoir of the *AIDA-1* and *EAE* genes in South Korea. A careful attention to control of these virulence factors is required in terms of public health and porcine diarrhea.

Zhang et al.[11] indicated that AIDA-1 (26.9%) and pAA (60%) are commonly found in the fimbrial isolates. However, in the present study only 5.4% and 4.1% of the 74 fimbrial isolates carried the *AIDA-1* and *pAA* genes, respectively. Especially, there were no fimbrial isolates carrying the *EAE* gene. Therefore, we could not determine the association between the fimbrial and non-fimbrial adhesins. Although AIDA-1, pAA, and EAE are considered as adhesion factors, which play a role in the attachment to porcine intestinal epithelial cell, produced by EPEC or EHEC[20,21,23,24,26]; however, the pathogenic significance of these factors has not been well explored[18,38,39]. In the present study, among the 42 non-fimbrial isolates, only seven isolates (16.7%) carried the genes encoding fimbrial adhesins, and 83.3% of the non-fimbrial isolates carried the genes for non-fimbrial adhesins that act only as adhesion factors. This higher prevalence suggests that the non-fimbrial adhesins alone is highly likely to induce porcine diarrhea by intestinal adherence of *E. coli*.

One hundred and seventy-two strains with one or more of the virulence genes were identified in the present study. These isolates were classified into several pathotypes, and ETEC was the major pathotype. Among the fimbrial adhesins, F4 and F18 were predominant. Among the fimbrial isolates, F4/LT/STb and F18/Stx2e were the most prevalent, although these virotypes were not significantly predominant. In addition, the *F4* and *F18* genes were strongly associated with LT or STb and Stx2e, respectively. The prevalence of the *AIDA-1*, *pAA*, and *EAE* genes, which encode non-fimbrial adhesins, was approximately 6%–7%. The association of non-fimbrial adhesins with fimbrial adhesins was difficult to identify. Especially, there was no association between EAE and the fimbrial adhesins. The current study suggests multiple virulence genes are associated with coliform diarrhea in piglets in Korea.

국문요약

Enterotoxigenic *Escherichia coli*는 신생 및 이유기 돼지 설사의 주요 원인체로서 전세계적으로 양돈산업에 큰 경제적 손실을 끼치고 있다. 그러나 현재 국내에는 이러한 *E. coli*가 보유하는 다양한 병원성유전자의 분포 및 특성에 대한 정보가 부족한 실정이다. 이에 본 연구에서는 2013년부터 2016년까지 국내 163개 양돈농장에서 이유기 설사증 개체로부터 면봉스왑 샘플을 채취하여 동일 농장의 개체일 경우 5개에서 10개 정도를 혼합한 후, MacConkey agar에 배양하여 최종 API 32E system을 통하여 동정하였다. 분리된 모든 균주에 대해서 3 가지의 다른 multiplex PCR을 수행하여 총 13 종의 병원성유전자의 분포를 확인하였다. 이를 통하여 총 172개의 최소 한가지 이상의 병원성 유전자를 가지는 *E. coli* 균주를 확인하였고, 그 결과 병원성 유전자의 분포는 (1) fimbrial adhesins (43.0%): F4 (16.9%), F5 (4.1%), F6 (1.7%), F18 (21.5%), and F41 (3.5%); (2) toxins (90.1%): LT (19.2%), STa (20.9%), STb (25.6%), Stx2e (15.1%), EAST1 (48.3%); and (3) non-

fimbrial adhesin (19.6%): EAE (14.0%), AIDA-1 (11.6%) and PAA (8.7%)로 나타났다. 결론적으로 본 연구결과는 국내 양돈농장의 이유기 설사증에 관연하는 *E. coli*는 다양한 종류의 병원성 유전자를 가지고 있으며 그러한 병원성 유전자의 조합도 매우 다양하게 분포하고 있음을 나타낸다.

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