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Virulence Genes of *Escherichia coli* Isolates from Piglets with Diarrhea in Korea

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Abstract: *Escherichia coli* is the most important etiological agent of diarrhea in young piglet. The aim of this research was to investigate the characteristics of serogroups, virulence genes and genetic diversity among *E. coli* isolated from young pigs with diarrhea in Korea. A total of 105 *E. coli* strains were isolated from rectal swabs and small intestinal contents of 1-6 weeks old pigs with diarrhea. About 47 (44.8%) of the isolates were one of the 11 serogroups, O2, O9, O11, O15, O103, O112, O121, O125, O139, O142 and O163. The most prevalent serogroup is O139 (10.5%) but 58 (55.2%) isolates were untypable. In pulsed field gel electrophoresis, the isolates were clustered in 40 groups of 50% similarity according to the dice similarity index and the most isolates in the same serogroups were classified differently. About 96 (91.4%) *E. coli* isolates possessed at least one toxin gene, *LT*, *STa*, *STb*, *Stx2e* and *EAST1*. The most prevalent toxin gene was *LT* (50 isolates) followed by STa (39 isolates) and STb (30 isolates). The 80 (76.2%) *E. coli* isolates also carried at least one fimbrial gene. The most prevalent fimbrial adhesin was F18 (37 isolates) followed by F6 (33 isolates) and F4 (29 isolates). Most isolates carring genes for fimbrial adhesins also possessed genes for toxin production. The most common associations were LT/STa/Stx2e/F18 (9 isolates), Stx2e/F18 (5 isolates), STa/Stx2e/F18 (4 isolates), LT/EAST1/F4 (4 isolates) and LT/STb/EAST1/F4/F6 (4 isolates). The results indicate the *E. coli* isolates from piglets with diarrhea in Korea showed a high genetic and phenotypic diversity.

Key words: Diarrhea, piglets, isolates, phenotypic diversity, genes, Korea

INTRODUCTION

Escherichia coli is the most important etiological agent of diarrhea in young piglets and is responsible for significant losses in large-scale farms worldwide. The main pathotype *E. coli* referred as Enterotoxigenic *E. coli* (ETEC) produces two major classes of enterotoxin, heat-Stable Toxin (STa, STb) and heat-Labile Toxin (LT) and also produce more than one fimbrial adhesion (F antigen). A second pathotype is Shiga Toxin-producing *E. coli* (STEC). Porcine STEC produce the shiga toxin 2e (Stx2e) which damages the vascular endothelium of the small intestine, subcutis and brain and ultimately leads to subcutaneous edema and neurological disorders (MacLeod *et al*., 1991). A new pathotype *E. coli* named Enteroaggregative *E. coli* (EAEC) is being increasingly recognized as an agent of diarrhea in young children in developing countries (Piva *et al*., 2003). EAEC isolates produce a low-molecular-weight and heat-stable plasmid encoded enteroaggregative heat-stable Enterotoxin 1 (EAST1). The gene encoding for EAST1 toxin is apparently not restricted to human EAEC and has also been detected in ETEC and STEC from humans and animals (Ngeleka *et al*., 2003; Osek, 2003). Enteric colibacillosis is the most common disease of piglets in Korea as in other countries. But little is known about serological and genetic characteristics of *E. coli* isolates. The aim of this research was to investigate the characteristics of serogroups, virulence genes and genetic diversity among *E. coli* isolated from young pigs with diarrhea in Korea.

A total of 105 *E. coli* strains were isolated from rectal swabs and small intestinal contents of 1-6 weeks old pigs with diarrhea. *E. coli* isolates were serogrouped by the Center for Infectious Disease, National Institute of Health, Republic of Korea using slide agglutination tests as described previously (Bettelheim *et al*., 2005; Bettelheim and Thompson, 1987). About 47 (44.8%) of the isolates were one of the 11 serogroups, O2, O9, O11, O15, O103, O112, O121, O125, O139, O142 and O163. The most prevalent serogroup is O139 (10.5%) but 58 (55.2%) isolates were untypable. Many researchers have reported that the limited serogroups of O8, O9, O20, O64, O101, O138, O139, O141, O147, O149 and O157 have been

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responsible for diarrhea in piglets (Blanco *et al*., 2004, 2006; Chen *et al*., 2004; Choi *et al*., 2001). However, the distribution and frequencies of serogroups can vary considerably from region to region.

Although, serogroup O139 was the predominat type in this study, the variety of serogroups was widespread in the country. In pulsed field gel electrophoresis, the isolates were clustered in 40 groups of 50% similarity according to the dice similarity index and the most isolates in the same serogroups were classified differently (Table 1).

MATERIALS AND METHODS

Polymerase chain reaction was carried out for detection of virulence genes (Table 2). Total 96 among 105 *E. coli* isolates passed at least one toxin gene, *STa*, *STb*, *Stx2e* and *EAST1*. The most prevalent toxin gene was *LT* (50 isolates) followed by *STa* (39 isolates) and *STb* (28 isolates) and thirty-three isolates were found 105 *E. coli* isolates possessed at least one toxin gene, *LT* to be carrying >2 toxin genes. One isolate possessed all the virulence genes tested in this study. Enterotoxins produced by *E. coli* isolated from piglets with diarrhea are important virulence factors that are directly responsible for induction of diarrhea in animals (Table 3).

Zhang *et al*. (2007) demonstrated that significant percentage (57.7%) among strains originating from diarrhea weaned piglets carried *LT* gene. Also, Frydendahl (2002) reported LT-positive isolates (47.6%) were more prevalent than STa (37.1%) and Stb-positive isolates (28.6%). On the other hand, Blanco *et al*. (2006) and Chen *et al*. (2004) reported that *STa* and *STb* toxin genes predominated in piglet with diarrhea.

Table 1: Distribution of O serogroup and PFGE patter types in 105 *Escherichia coli* isolates from diarrhoeic piglets

Serogroup	Total no. of isolates	PFGE patter type (No. of isolates included)
O2	4	X-6 (1), X-16 (1), X-31 (1), X-35 (1)
O9	4	X-8 (2), X-23 (1), X-30 (1)
O11	1	X-20 (1)
O15	1	X-2 (1)
O103	5	X-17 (5)
O112	6	X-4 (5), X-5 (1)
O121	5	X-6 (1), X-11 (1), X-16 (1), X-27 (1), X-31 (1)
O125	1	X-36 (1)
O139	11	X-1 (1), X-12 (1), X-18 (2), X-19 (1), X-23 (1), X-25 (1), X-32 (1), X-35 (2), X-36 (1)
O142	5	X-3 (1), X-5 (1), X-18 (1), X-20 (1), X-35 (1)
O163	4	X-11 (2), X-13 (1), X-21 (1)
ONT[a]	58	X-4 (9), X-5 (4), X-6 (1), X-7 (1), X-9 (1), X-10 (1), X-11 (5), X-12 (1), X-14 (2), X-15 (2), X-18 (1), X-20 (1), X-21 (1), X-22 (1), X-23 (3), X-24 (1), X-25 (1), X-26 (1), X-28 (1), X-29 (3), X-33 (1), X-34 (1), X-35 (11), X-37 (1), X-38 (1), X-39 (1), X-40 (1)
Total	105	

[a]ONT; O antigen not typable

Table 2: Primer sequences used to amplify target genes coding for virulence factors and predicted lengths of PCR amplification products

Target genes	Oligonucleotide sequences	Fragment size (bp)	Accession number	References
LT	5'-ATGATATATAAGTTTTCCTCGATG-3' 5'-TCATAATTCATTCCGAATTCTGTT-3'	798	S60731	This study
STa	5'-ATGACGGGAGGTAACATGAAAAA-3' 5'-TTAATAACATCCAGCACAGGCAG-3'	234	M58746	This study
STb	5'-ATGAAAAAGAATATCGCATTTCTTC-3' 5'-TTAGCATCCTTTTGCTGCAACCA-3'	216	AY028790	This study
Stx2e	5'-ATGTATATGAAGTGTATATTGTTAA-3' 5'-TCATTCACCAGTTGTATATAAAGA-3'	966	M36727	This study
EASTI	5'-ATGTGGCTGGCCGAAAATGAAG-3' 5'- TGGATAAGCGAAGAACGTGGCA-3'	378	S81691	This study
F4 (*K88*)	5'-GGTGATTTCAATGGTTCGGTC-3' 5'-ATTGCTACGTTCAGCGGAGCGC-3'	773	M29374	Franklin *et al*. (1996)
F5 (*K99*)	5'-ATGAAAAAAACACTGCTAGCTATT-3' 5'-TTACATATAAGTGACTAAGAAGGA-3'	546	M35282	This study
F6 (*P987*)	5'-CTGAAAACAACACCAGCCAG-3' 5'-GGTGGTTCCGATGTATGCTT-3'	419	M35257	This study
F17	5'-ATGACAAATTTTTATAAGGTCTTTC-3' 5'-TTACTGATAGGAAAATGTAAATGTT-3'	1,035	AF055313	This study
F18	5'-GGTACTGTTGCACCAAGCGG-3' 5'-CGACGCCTTAACCTCCTGCCCC-3'	510	M61713	Blanco *et al*. (2006)
F41	5'-ATGAAATGTCAAGGTGATTATTTTA-3' 5'-TTAACTATAAATAACGGTGATAGTC-3'	858	X14354	This study

Table 3: Distribution of virulence genes of 105 *E. coli* isolates from piglets with diarrhea

	Toxin genes											
Fimbria	No toxin	*LT* only	*STa* only	*STb* only	*Stx2e* only	*EAST1* only	*LT/STa*	*LT/STb*	*LT/Stx2e*	*LT/EAST1*	*STa/Stx2e*	*STb/Stx2e*
No fimbria	9	2	1	1	5	-	-	-	2	1	1	-
F4 only	-	1	-	-	-	1	-	1	-	4	-	-
F6 only	10	-	-	-	-	-	-	-	3	-	-	-
F17 only	-	-	-	-	1	-	-	-	-	-	-	-
F18 only	-	-	1	-	5	1	-	-	1	-	4	1
F4/F6	-	1	-	-	-	-	-	1	-	-	-	-
F4/F17	-	-	-	-	-	-	-	1	-	-	-	-
F4/F18	-	-	2	-	-	-	-	-	-	-	-	-
F5/F18	-	-	-	-	-	-	-	-	-	-	-	-
F6/F17	1	-	-	-	-	-	-	-	-	-	-	-
F6/F18	-	-	-	-	-	-	1	-	1	-	-	1
F17/F18	-	1	-	-	-	-	-	-	-	-	-	-
F4/F6/F18	-	-	-	1	-	-	-	-	-	-	-	-
F5/F6/F17	1	-	-	-	-	-	-	-	-	-	-	-

	Toxin genes											
Fimbria	*STa/ STb*	*LT/ STa/ STb*	*LT/ STa/ Stx2e*	*LT/ STb/ EAST1*	*LT/ Stx2e/ EAST1*	*STa/ STb/ Stx2e*	*LT/STa/ STb/ EAST1*	*LT/STa/ Stx2e/ EAST1*	*LT/STa/ Stx2e/ EAST1*	*LT/STb/ Stx2e/ EAST1*	*STa/STb/ Stx2e/ EAST1*	*LT/STa/ STb/Stx2e/ EAST1*
No fimbria	-	-	2	-	-	-	-	-	-	-	-	1
F4 only	-	1	-	1	-	-	1	1	1	2	-	-
F6 only	-	-	-	-	-	-	-	-	-	-	-	-
F17 only	-	-	-	-	-	-	-	-	-	-	1	-
F18 only	1	-	9	-	-	2	-	-	-	-	-	-
F4/F6	-	-	-	4	1	-	3	1	-	-	-	-
F4/F17	-	-	-	-	-	-	-	-	-	-	-	-
F4/F18	-	-	-	-	-	-	-	-	-	-	-	-
F5/F18	-	-	1	-	-	-	-	-	-	-	-	-
F6/F17	-	-	-	-	-	-	-	-	-	-	-	-
F6/F18	3	-	-	-	-	-	-	-	-	-	-	-
F17/F18	-	-	-	-	-	1	-	-	-	-	-	-
F4/F6/F18	-	-	-	-	-	-	-	-	-	-	-	-
F5/F6/F17	-	-	-	-	-	-	-	-	-	-	-	-

RESULTS AND DISCUSSION

Eighty among 105 *E. coli* isolates also carried at least one fimbrial gene. The most prevalent fimbrial adhesin was F18 (37 isolates), followed by F6 (33 isolates) and F4 (29 isolates). But 12 of 37 F18-positive isolates were possessed either F4-F6 or F17. The percentage of F4-positive *E. coli* strains isolated from pigs described by other investigators was usually higher than that found in this study. Wilson and Francis (1986) demonstrated that a significant percentage (72%) among strains originating from diarrheal weaned piglets carried the *F4* genes. Also Nagy and Fekete (1999) reported the presence of F4 fimbria in 61% of *E. coli* strains tested. On the other hand, Ojeniyi *et al*. (1994) found only 25.8% of isolates originated from pigs with postweaning diarrhea showed F4-positive. Similarly, Chen *et al*. (2004) reported that 9.8% of 215 *E. coli* isolates from pigs with PED were F4-positive. This may be related to the widespread use of vaccines contained F4 and F18 incorporating fimbriated strains (Fairbrother *et al*., 1988; Harel *et al*., 1991; Soderlind *et al*., 1988). In Korea, the inactivated vaccines for sows have been used countrywide. These vaccines contained *E. coli* whole cells with F4 and F8 in a water in oil emulsion. Also, it is suggest that the prevalence of ETEC strains expressing fimbriae can be age-related and also vary with geographic locations. Garabal *et al*. (1997) and Wilson and Francis (1986) reported F6 fimbria have been the most frequently detected isolates from suckling piglets with diarrhea. Dean *et al*. (1989) also demonstrated infection with ETEC strains carrying fimbria is age-related. Ngeleka *et al*. (2003) reported that EAST1 pathotype was common among *E. coli* isolates from both diarrheic and non-diarrheic piglets. A possible explanation for the existence of EAST1-producing *E. coli* in healthy piglets is that some healthy piglets can be associated with asymptomatic infection or that these *E. coli* can only cause diarrhea in piglets in the presence of other virulence factors such as enterotoxins (LT, STa or STb) and specific colonization factors.

CONCLUSION

In this study, 22 among 24 isolates carrying EAST1 also possessed another virulence factor. Most isolates carrying genes for fimbrial adhesins also possessed genes for toxin production. The most common associations were LT/STa/Stx2e/F18 (9 isolates), Stx2e/F18 (5 isolates), STa/Stx2e/F18 (4 isolates), LT/EAST1/F4 (4 isolates) and LT/STb/EAST1/F4/F6 (4 isolates).

Consequently, these results indicate the *E. coli* isolates from piglets with diarrhea in Korea showed a high genetic and phenotypic diversity.

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