

Special Article - *Escherichia coli*

Virulence-associated Genes and Antimicrobial Resistance of *Escherichia coli* Isolated From Post-weaning Piglets with Diarrhea in Korea

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Received: November 27, 2018; Accepted: December 18, 2018; Published: December 26, 2018

Abstract

Post-weaning diarrhea and/or porcine edema disease is caused by pathogenic *Escherichia coli*. In this study, 139 *E. coli* isolates were collected from post-weaning piglets with diarrhea and/or edema disease between 2013 and 2016 in Korea. Virulence (fimbria and toxin) genes and antimicrobial resistance were evaluated in the isolates. Among the 139 *E. coli* isolates, the F4ac fimbriae gene was observed in 7.2% of isolates, and the F18 variant fimbria genes were found to be F18 (12.9%), F18ab (33.1%), and F18ac (20.1%). The various fimbriae gene combinations were F4/F18/F41 (0.7%), F4ac/F18ab (0.7%), and F4ac/F18ac (1.4%) in the 139 *E. coli* isolates. A total of 111 enterotoxigenic *E. coli* (ETEC) isolates were detected, and the most prevalent toxin genes were identified as STa (35.9%) and STb (35.2%). Additionally, among 111 ETEC isolates, 79 isolates harbored the Stx2e gene. All 139 *E. coli* isolates were resistant to ceftiofur and sulphafurazole. In contrast, all isolates were susceptible to amikacin, vancomycin, lincomycin, and tircarcillin/clavulanic acid. All of the ampicillin, ceftiofur, chloramphenicol, imipenem, kanamycin, sulphafurazole, tetracycline, cephalosporin, and ticarcillin-resistant isolates between 2013 and 2015, and the ampicillin, ceftiofur, cephalothin, chloramphenicol, streptomycin, sulphafurazole, tetracycline, and ticarcillin-resistant isolates from 2016 showed high resistance to the carbapenem, cephem, penicillins, phenicols, sulfas, and tetracyclines class and to the aminoglycosides, cephem, penicillin, phenicols, sulfas, and tetracyclines class. These results suggest that *E. coli* disease in piglets may not be associated with a single toxin or a major gene combination, but with more varied and complex toxins and their combinations.

Keywords: Antimicrobial Resistance; *Escherichia coli*; Piglets; Diarrhea

Introduction

Escherichia coli (*E. coli*) is an important pathogen in post-weaning diarrhea and/or edema disease, and is commonly found in pigs' intestinal tracts on a wide variety of farms [1]. Various *E. coli* types adhere to the intestinal tracts via fimbriae, including F4, F5, F6, F18, and F41, and produce toxins, which include LT, STa, STb, and shiga toxin [2]. The fimbriae factor, F4⁺ *E. coli*, is associated with neonatal and post-weaning diarrhea, whereas F18⁺ *E. coli* causes post-weaning diarrhea and/or edema disease in piglets [3]. F4⁺ and F18⁺ *E. coli* exist as several variant types. Among the F4⁺ *E. coli*, there are three variants, F4ab, F4ac, and F4ad, in which fimbriae share a common 'a' epitope and type-specific epitope 'b', 'c', and/or 'd' determinants [4-7]. F18⁺ *E. coli* has two variants, namely F18ab and F18ac. Both fimbriae are colonization factors for enterocyte receptors, and F18ac is found mostly in pigs with edema diseases via enterotoxigenic *E. coli* [8-10]. Several studies have investigated the variant F4⁺ and F18⁺ fimbriae factors of *E. coli* isolated from pigs with diarrhea and/or edema disease [11-13], including those in Korea [14,15].

Most pathogenic *E. coli* causing porcine diarrhea and/or edema disease are characterized by antimicrobial resistance. The *E. coli* isolates from pigs, cattle and poultry are often resistant to two or more

antimicrobials, including sulfamethoxazole (Sx), tetracycline (Te), streptomycin (S), ampicillin (Am), spectinomycin (Sp), apramycin (Ap), trimethoprim-sulfonamide (TMP/SMX), and neomycin (Nm) [16,17]. Recently, the frequency of antimicrobial resistance among *E. coli* has increased markedly, presumably owing to extensive and indiscriminate antimicrobial use, which has caused difficulty in treating pathogenic *E. coli* infections in animals and humans. Therefore, an increase in the incidence of outbreaks of severe *E. coli*-associated diarrhea and/or edema disease has been observed worldwide, and several studies have investigated antimicrobial resistance against *E. coli* isolates [2,18-21].

The present study's objective was to evaluate the distribution of types of pathogenic *E. coli* isolates from pigs with diarrhea and/or edema disease, isolated between 2013 and 2016 in Korea, and examine the characteristics of their virulence genes, including fimbrial and toxin genes. We applied nucleotide primers and conditions for polymerase chain reaction (PCR) to detect these fimbriae and toxins using a previously reported method and/or modified method [4,10]. Another objective of the present study was to examine antimicrobial resistance and determine its genetic basis in the isolates.

Table 1: Detection of virulence genes from *E. coli* isolates.

Target gene	Primer sequences (5' to 3')	Product size
F4	F: TGAATGACCTGACCAATGGTGAACC	484bp
	R: GCGTTTACTCTTTGAATCTGTCCGAG	
F5	F: GCGACTACCAATGCTTCTGCGAATAC	230bp
	R: GAACCAGACCAGTCAATACGAGCA	
F6 (987P)	F: GCCAGTCTATGCCAAGTGGATACTTC	391bp
	R: GTTTGTATCAGGATCCCTGTGGTGG	
F18	F: TGGCACTGTAGGAGATACCATTGAGC	334bp
	R: GGTGGTGGACCTTTTCAGTTGAGCAG	
F41	F: TTAGCAGCGAAGATGAGTGATGGG	515bp
	R: GTACTACCTGCAGAAACACCAGATCC	
LT	F: ACGGCGTTACTATCCTGTCTATGTGC	275bp
	R: TTGGTCTCGTCCAGATATGTGATTCT	
STa	F: GTCAGTCAACTGAATCACTTGACTCT	152bp
	R: CATGGAGCACAGGCAGGATTACAACA	
STb	F: GCTACAAATGCCTATGCATCTACACA	125bp
	R: CATGCTCCAGCAGTACCATCTCTAAC	
Stx2e (Pig)	F: CGGTATCCTATTCCAGGAGTTTACG	599bp
	R: GTCTTCCGGCGTCATCGTATAAACAG	

Zhang, et al. 2007. [23].

Methods

Bacterial strains and growth conditions

A total of 139 pathogenic *E. coli* strains were isolated from fecal and intestinal samples taken from pigs diagnosed with diarrhea and edema disease at a Korean pig farm between 2013 and 2016. A 10-fold dilution of fecal and homogenized intestinal samples were inoculated on MacConkey agar (Oxoid, UK). The colonies were identified using standard biochemical procedures [22] and PCR analysis [15]. The 139 *E. coli* isolates were routinely grown in Luria-Bertani (LB) agar and/or broth (Affymetrix USB, CA, USA). Mueller-Hinton agar (Becton, Dickinson and Company, NY, USA) was used for antimicrobial assessment of the 139 *E. coli* isolates.

Detection of virulence gene using PCR

The 139 *E. coli* isolates were grown on LB broth at 37°C for 24h. DNA extraction was performed by boiling at 100°C for 10min and centrifugation at 10,000xg for 5min. The supernatant was used as the DNA template for PCR amplification. Multiplex PCR to detect the *E. coli* virulence factor gene was performed according to methods described in a previous report [23]. In brief, Multiplex PCR was performed using the PCR premix kit (AccuPower[®] Multiplex PCR PreMix, Daejeon, Bionner) and virulence gene-specific primers (Table 1). The PCR amplification process was performed with a thermal cycler (Applied Biosystems, CA, USA) under the following conditions: Initial denaturation (95°C, 15min); 25 cycles of denaturation (95°C, 30sec), annealing (63°C, 90sec), and extension (72°C, 90sec); final extension (72°C, 10min). In the reaction's final step, the PCR mixture was cooled to 4°C. The PCR products were analyzed by electrophoresis on a 3% agarose gel containing Red Safe[™] (iNtRON Biotechnology, Seongnam, Korea), and detected using an ultraviolet (UV) visualization system. According to the

Table 2: Toxin and fimbriae adhesin genes detected in 139 *E. coli* isolates.

Fimbriae adhesin genes	Number of strains	Toxin gene				
		Number (and %)				
		LT	STa	STb	Stx2e	None
F4	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
F4ac	10	5 (50.0)	2 (20.0)	8 (80.0)	2 (20.0)	0 (0)
F6	2	2 (100.0)	0 (0)	2 (100.0)	0 (0)	0 (0)
F18	18	5 (27.8)	6 (33.3)	2 (11.1)	13 (72.2)	2 (11.1)
F18ab	46	17 (37.0)	17 (37.0)	7 (15.2)	31 (67.4)	4 (8.7)
F18ac	28	6 (21.4)	15 (53.6)	10 (35.7)	20 (71.4)	2 (7.1)
F41	3	1 (33.3)	0 (0)	0 (0)	0 (0)	2 (66.7)
F4+F18+F41	1	0 (0)	0 (0)	1 (100.0)	0 (0)	0 (0)
F4ac+F18ab	1	0 (0)	1 (100.0)	0 (0)	0 (0)	0 (0)
F4ac+F18ac	2	1 (50.0)	2 (100.0)	2 (100.0)	0 (0)	0 (0)
None	28	2 (7.1)	7 (25.0)	17 (60.7)	13	0 (0)

method of Zhang, et al [23], the virulence genes (F4, F5, F6, F18, F41, LT, STa, STb, and Stx2e) were confirmed by the band sizes of the PCR product (Table 1).

PCR for detection of F4⁺ or F18⁺ *E. coli* variants

To determine the variants of the fimbriae adhesin gene and variants type gene (F4, F4ac, F4ad, F18, F18ab, F18ac, and F41) of the 139 *E. coli* isolates, specific primers were used as described previously [4,10]. Multiplex PCR was performed using the PCR premix kit (AccuPower[®] Multiplex PCR PreMix) with a total reaction volume of 20µl, which consisted of 3µl of template DNA, 1µl of 10pmol/l primers (F4, F4ac, F4ad, F18, F18ab, F18ac, and F41), and 17µl of distilled water. The F4 (K88) gene variants (F4ab and F4ad) primers and PCR conditions used were as described in a previously reported method [4]. We detected the F18 gene variants according to the modified PCR condition method and used F18 gene variant detection primers in accordance with the report by Cheng, et al [10]. In brief, we modified the PCR amplification process with a thermal cycler (Applied Biosystems, CA, USA) under the following conditions: initial denaturation (94°C, 3min); 25 cycles of denaturation (92°C, 30sec), annealing (50°C, 30sec), and extension (72°C, 60sec); final extension (72°C, 10min) for F18ab and F18ac (fedA1, fedA2, fedA3). In the final step of each PCR reaction, the mixture was cooled to 4°C, and the products were analyzed by electrophoresis on a 1.5% agarose gel containing Red Safe[™] (iNtRON Biotechnology). The PCR products were detected using a UV visualization system.

Antimicrobial susceptibility testing

The antimicrobial susceptibilities of all *E. coli* isolates were assessed using the disk-agar method, as standardized by the Clinical and Laboratory Standards Institute (CLSI) [24]. The isolates were tested against a panel of 23 antimicrobials: Amikacin (An), ampicillin (Am), azithromycin (Azm), cefamandole (Ma), cefoxitin (Fox), ceftriaxone (Cro), cephalothin (Cf), chloramphenicol (C), ciprofloxacin (Cip), gentamicin (Gm), imipenem (Ipm), kanamycin (K), nalidixic acid (Na), streptomycin (S), tetracycline (Te), ticarcillin/clavulanic acid (Tim), ticarcillin (Tic), vancomycin (Va) (BD, NJ, USA) and ceftiofur (Ef), cephalixin (Cl), cephalozin (Kz), lincomycin (My), sulphafurazole (Sf), and sulphamethoxazole (Sx) (Thermo Scientific[™]

Table 3: Antimicrobial resistance rates of 139 *E.coli* isolates collected from Korean pig farms in 4 periods.

Antimicrobial	Collection period; number (and %) resistant							
	2013		2014		2015		2016	
	(n = 60)	(n = 10)	(n = 10)	(n = 10)	(n = 51)	(n = 51)	(n = 18)	(n = 18)
Aminoglycosides								
Amikacin	0	0	1	-10	0	0	2	-11.1
Gentamicin	43	-71.7	3	-30	20	-39.2	13	-72.2
Kanamycin	42	-70	7	-70	29	-56.9	11	-61.1
Streptomycin	56	-93.3	6	-60	39	-76.5	17	-94.4
Carbapenem								
Imipenem	56	-93.3	10	-100	46	-90.2	1	-5.6
Cephems								
Cefamandole	39	-65	7	-70	17	-33.3	5	-27.8
Cefoxitin	19	-31.7	1	-10	5	-9.8	3	-16.7
Ceftiofur	60	-100	10	-100	51	-100	18	-100
Ceftriaxone	4	-6.7	0	0	1	-2	0	0
Cephalothin	46	-76.7	4	-40	31	-60.8	9	-50
Cephalexin	29	-48.3	2	-20	12	-23.5	14	-77.8
Cephazolin	59	-98.3	10	-100	44	-86.3	5	-27.8
Glycopeptides								
Vancomycin	0	0	1	-10	6	-11.8	0	0
Lincosamides								
Lincomycin	0	0	1	-10	5	-9.8	0	0
Macrolides								
Azithromycin	14	-23.3	1	-10	14	-27.5	0	0
Penicillins								
Ampicillin	55	-91.7	9	-90	43	-84.3	17	-94.4
Ticarcillin/Clavulanic acid	5	-8.3	0	0	4	-7.8	1	-5.6
Ticarcillin	51	-85	8	-80	45	-88.2	16	-88.9
Phenicol								
Chloramphenicol	59	-98.3	10	-100	43	-83.4	17	-94.4
Quinolones								
Ciprofloxacin	36	-60	2	-20	16	-31.4	7	-38.9
Nalidixic acid	46	-76.7	3	-30	19	-37.3	11	-61.1
Sulfas								
Sulphafurazole	60	-100	10	-100	50	-98	18	-100
Sulphamethoxazole	42	-70	6	-60	30	-58.8	13	-72.2
Tetracyclines								
Tetracycline	52	-86.7	8	-80	37	-72.5	17	-94.4

Oxoid™, Hampshire, UK). The results were obtained after incubating the samples for 24h at 37°C and were interpreted according to the CLSI [24]. The resistant types were considered identical if they were similarly susceptible to all the agents tested. The quality control strains included *E. coli* ATCC25922 (American Type Culture Collection).

Results

Fimbrial adhesins and virulence genes

The fimbrial adhesin genes, F4, F6, F18, and F41, were detected in

the 139 *E. coli* isolates, and the results are shown in Table 2. Among the 139 *E. coli* isolates collected between 2013 and 2016, 111 *E. coli* isolates (79.9%) exhibited one or more fimbrial adhesin genes. Among the 111 *E. coli* isolates, 10 (9.0%), two (1.8%), 18 (16.2%), 46 (41.4%), 28 (25.2%), and three (2.7%) isolates were detected as F4ac, F6, F18, F18ab, F18ac, and F41, respectively. Various fimbrial adhesin gene combinations were identified in 4/111 *E. coli* isolates: One (0.9%), one (0.9%), and two (1.8%) isolates were detected as F4/F18/F41, F4ac/F18ab, and F4ac/F18ac, respectively of the 139 *E. coli* isolates, 28 *E. coli* (20.1%) were shown to have only a toxin gene, without fimbrial adhesin genes. Ten of the *E. coli* isolates with F4ac had several toxin genes, including LT (50%), STa (20%), STb (80%), and Stx2e (20%). Of the 92 *E. coli* isolates with the F18, F18ab, and F18ac adhesin genes, a number were positive for LT (27.8, 37.0, and 21.4 %), STa (33.3, 37.0, and 53.6 %), STb (11.1, 15.2, and 35.7 %), Stx2e (72.2, 67.4, and 71.4 %), and negative for the toxin gene (11.1, 8.7, 7.1 %). *E. coli* isolates carrying the F4/F18/F41 (1/139), F4ac/F18ab (1/139), and F4ac/F18ac (2/139) combinations were positive for LT (0, 0, and 50 %), STa (0, 100, and 100 %), and STb (100, 0, and 100 %), respectively.

Antimicrobial susceptibility

Among the 139 *E. coli* isolates, 60, 10, 51, and 18 strains were isolated in 2013, 2014, 2015, and 2016, respectively. The 139 *E. coli* isolates were analyzed for antimicrobial susceptibility (Table 3). Among the 139 *E. coli* isolates, all were resistant to one or more of the antimicrobials tested, regardless of the collection date. The antimicrobial susceptibility of the 60 *E. coli* isolates from 2013 exhibited higher resistance to Am, C, Ef, Ipm, Kz, S, Sf, and Te, and the 10 *E. coli* isolates from 2014 exhibited higher resistance to Am, C, Ef, Ipm, Cf, and Sf (resistance rate ≤85%) compared to the other antimicrobial agents (resistance rate ≥85%). The antimicrobial susceptibility of the 51 *E. coli* isolates collected in 2015 exhibited higher resistance to Ef, Ipm, Kz, Sf, and Tic, and the 18 *E. coli* isolates collected in 2016 exhibited higher resistance to Am, C, Ef, S, Sf, Te, and Tic (resistance rate ≤85%) compared to the other antimicrobial agents (resistance rate ≥85%). All *E. coli* isolates collected between 2013 and 2016 were shown to have 100% rates of resistance to Ef and Sf. By contrast, all isolates showed susceptibility to An, My, Tim, and Va (resistance rate 0-11.8%).

In total, 23 antimicrobial resistance patterns were observed in the 139 *E. coli* isolates (Table 4). Of the 121 *E. coli* isolates collected between 2013 and 2015, 28 (2.1% of 60) from 2013, five (2.0% of 10) from 2014, and 12 (4.1% of 51) from 2016 had the most frequent resistance phenotype, AmEfCIpmKSfTeKzTic (type 113). Among the 18 *E. coli* isolates from 2016, the most frequent resistance types were AmEfGmSSfSxTeClTic (type 116) and AmEfCfCSSfTeTic (type 119) (six isolates each; 3%).

Discussion

E. coli is distributed across a wide variety of farms and wild animals; it has been identified as the cause of disease and associated with outbreaks [25-27]. In this study, we isolated 139 *E. coli* strains from pigs with diarrhea and/or edema disease in Korea between 2013 and 2016, investigating the fimbriae adhesin, virulence genes, and antimicrobial susceptibility.

The toxin-producing *E. coli* strains with various fimbriae adhesin

Table 4: Patterns of antimicrobial resistance of the isolates.

Year of collection	Type	Resistance	
		Pattern ^a	Number (and %) of isolates (n=139)
2013	1	AmAzmMaFoxEfCfCCipGmlpmKNaSSfSxTeClTimKzTic	1 -0.7
	2	AmAzmMaFoxEfCfCCipGmlpmKNaSSfSxTeClTimKz	1 -0.7
	3	AmAzmMaFoxEfCfCCipGmlpmKNaSSfSxTeClKzTic	1 -0.7
	4	AmMaFoxEfCfCCipGmlpmKNaSSfSxTeClTimKzTic	1 -0.7
	5	AmAzmMaFoxEfCfCCipGmlpmKNaSSfTeClTimKzTic	1 -0.7
	7	AmMaFoxEfCfCCipGmlpmKNaSSfSxTeClKzTic	4 -2.9
	9	AmMaFoxEfCfCCipGmlpmKNaSSfSxTeClTimKzTic	1 -0.7
	11	AmMaFoxEfCfCCipGmlpmKNaSSfSxTeClKz	1 -0.7
	12	AmMaEfCroCfCCipGmlpmKNaSSfSxClKzTic	1 -0.7
	13	AmFoxEfCroCfCCipGmlpmKNaSSfSxClKzTic	1 -0.7
	15	AmAzmMaEfCCipGmlpmKNaSSfSxTeKzTic	2 -1.4
	17	AmAzmEfCfCCipGmlpmKNaSSfSxTeClKz	1 -0.7
	19	AmAzmEfCfCCipGmlpmNaSSfSxTeClKzTic	1 -0.7
	20	AmMaFoxEfCfCCipGmlpmNaSSfSxTeClKz	2 -0.7
	22	AmMaFoxEfCfCCipGmlpmKNaSSfSxTeKzTic	1 -0.7
	25	AmEfCfCCipGmlpmKNaSSfSxTeClKzTic	1 -0.7
	26	AmAzmMaEfCfCCipGmlpmNaSSfSxKzTic	1 -0.7
	28	AmAzmEfCfCCipGmlpmKNaSSfSxTeKzTic	1 -0.7
	29	AmMaEfCfCGmlpmKNaSSfSxTeKzTic	1 -0.7
	31	AmMaEfCCipGmlpmKNaSSfSxTeKzTic	1 -0.7
	32	AmFoxEfCfCCipGmlpmNaSSfSxTeClKz	1 -0.7
	34	MaFoxEfCroCfCiplpmKSSfSxTeClKzTic	2 -1.4
	38	AmAzmMaEfCfCCiplpmNaSSfSxKzTic	1 -0.7
	40	AmAzmEfCfCGmlpmKSSfSxTeKzTic	1 -0.7
	42	AmMaEfCfCCiplpmNaSSfSxClKzTic	1 -0.7
	43	AmMaEfCfCiplpmKNaSSfTeClKzTic	1 -0.7
	44	AmMaEfCfCipGmlpmKNaSSfTeKzTic	1 -0.7
	45	AmMaEfCCiplpmKNaSSfSxTeKzTic	1 -0.7
	47	AmEfCfCCipGmlpmKNaSSfTeClKz	1 -0.7
	51	AmEfCCipGmlpmKNaSSfSxTeKzTic	1 -0.7
	52	AzmFoxEfCfCGmlpmNaSfSxTeClKzTic	1 -0.7
	56	AmAzmEfCGmlpmKNaSSfTeKzTic	1 -0.7
	57	AmMaEfCroCfCiplpmNaSSfSxTeKzTic	1 -0.7
	59	AmMaEfCfCGmlpmKNaSfTeKzTic	1 -0.7
	60	AmMaEfCfCGmlpmKSSfTeKzTic	1 -0.7
	61	AmMaEfCfCGmlpmNaSSfTeKzTic	1 -0.7
	62	AmMaEfCfCGmlpmSSfSxTeKzTic	2 -1.4
	63	AmMaEfCfCiplpmKSSfTeClKzTic	1 -0.7
	64	AmMaEfCfCiplpmKSSfSxTeClKzTic	1 -0.7
	67	AmEfCCipGmlpmKNaSSfTeKzTic	1 -0.7
	68	AmEfCCipGmKNaSSfSxTeKzTic	1 -0.7
	72	AmMaEfCfCiplpmKSSfTeKzTic	1 -0.7
	73	AmMaEfCfCiplpmNaSSfTeKzTic	1 -0.7
	75	AmMaEfCGmKSSfSxTeKzTic	1 -0.7
	76	AmEfCfCGmlpmKSSfTeClKz	1 -0.7
78	AmEfCfCGmlpmNaSSfTeKzTic	2 -1.4	
80	AmEfCCipGmlpmKNaSSfSxTic	1 -0.7	
90	AmEfCfCGmNaSSfTeKzTic	1 -0.7	
98	EfCfCCiplpmSSfSxClKzTic	1 -0.7	
102	AmMaEfCipMSSfTeKzTic	1 -0.7	
111	EfCCiplpmKNaSSfSxKz	1 -0.7	
115	AmEfCKSSfTeKzTic	1 -0.7	

2014	6	AmAzmEfCCipGmlpmKNaSSfSxTeCIVaMyKzTic	1	-0.7
	12	AmMaFoxEfCfCCipGmlpmKNaSSfSxClKzTic	1	-0.7
	53	EfCfCCipGmlpmKNaSSfSxTeKzTic	1	-0.7
	104	AmEfCfClpmSSfTeKzTic	1	-0.7
	108	AmEfClpmKSfSxTeKzTic	2	-1.4
	109	AmEfClpmNaSSfSxTeKz	1	-0.7
	113	AmEfClpmKSfTeKzTic	1	-0.7
	114	AmEfClpmSSfTeKzTic	1	-0.7
	121	EfCfClpmKSfSxKz	1	-0.7
2015	10	AmAzmMaFoxEfCfCCiplpmKNaSSfSxCITimKz	1	-0.7
	12	AmMaFoxEfCfCCipGmlpmKNaSSfSxClKzTic	1	-0.7
	14	AmAzmMaEfCfClpmSSfSxTeCITimVaMyTic	1	-0.7
	18	AmAzmEfCfCCipGmlpmKNaSSfSxTeKzTic	1	-0.7
	23	AmMaEfCfCCipGmlpmKNaSSfSxTeKzTic	1	-0.7
	24	AmFoxEfCfCCipGmlpmKNaSSfSxTeKzTic	1	-0.7
	27	AmAzmMaEfCfCGmlpmKSSfSxTeKzTic	1	-0.7
	30	AmMaEfCfGmlpmKSSfTeCITimVaMyTic	1	-0.7
	33	AmEfCfCCipGmlpmKNaSSfSxTeKzTic	1	-0.7
	37	AmAzmMaFoxEfCfCCiplpmNaSfSxTeKzTic	1	-0.7
	39	AmAzmEfCfCGmlpmKNaSSfSxKzTic	1	-0.7
	41	AmAzmEfCCipGmlpmKNaSSfSxKzTic	1	-0.7
	49	AmEfCfCGmlpmNaSSfSxTeVaKzTic	1	-0.7
	50	AmEfCfCGmSSfSxTeCITimVaMyTic	1	-0.7
	53	EfCfCCipGmlpmKNaSSfSxTeKzTic	1	-0.7
	54	AmAzmMaEfCfClpmSSfSxTeKzTic	1	-0.7
	55	AmAzmEfCCipGmlpmKNaSfTeKzTic	1	-0.7
	58	AmMaEfCfCCiplpmSSfSxTeKzTic	1	-0.7
	66	AmEfCfCCiplpmKNaSSfTeKzTic	1	-0.7
	69	AmAzmEfCGmlpmSSfSxTeKzTic	1	-0.7
	70	AmAzmEfClpmKSSfSxTeKzTic	1	-0.7
	71	AmMaEfCfClpmKSSfSxKzTic	1	-0.7
	74	AmMaEfCfClpmSSfSxTeKzTic	1	-0.7
	77	AmEfCfCGmlpmKSfSxTeKzTic	1	-0.7
	79	AmEfCfCSSfSxTeCIVaMyTic	1	-0.7
	82	AmEfCipKNaSSfSxTeClKzTic	1	-0.7
	83	FoxEfCroCfCiplpmKNaSSfKzTic	1	-0.7
	84	EfCfCGmlpmKNaSfSxTeKzTic	1	-0.7
	85	AmAzmEfClpmKSSfTeKzTic	1	-0.7
	86	AmMaEfCfCGmSfTeClKzTic	1	-0.7
	87	AmMaEfCfClpmKSfTeKzTic	1	-0.7
	88	AmMaEfCfClpmSfTeClKzTic	1	-0.7
	89	AmMaEfClpmSSfSxTeKzTic	1	-0.7
	91	AmEfCfClpmKSSfSxKzTic	1	-0.7
	92	AmEfCGmlpmNaSfSxTeKzTic	1	-0.7
	95	AmEfClpmKSSfSxTeKzTic	1	-0.7
	99	EfCfClpmSSfTeCIVaMyTic	1	-0.7
	100	AmMaEfCfClpmSfTeKzTic	1	-0.7
	106	AmEfCfCiplpmSfSxClKzTic	1	-0.7
	107	AmEfClpmKSSfTeKzTic	1	-0.7
	110	AzmEfClpmKNaSSfSxKz	1	-0.7
	112	AmMaEfClpmSSfClKz	1	-0.7
	114	AmEfClpmSSfTeKzTic	2	-1.4
	115	AmEfCKSSfTeKzTic	1	-0.7
	117	AmEfClpmKSSfTeKzTic	1	-0.7
118	AmEfCfCGmlpmKzTic	1	-0.7	
120	AmEfClpmSSfKzTic	1	-0.7	
122	AzmEfGmlpmKSSf	1	-0.7	
123	EfGmlpmSSfTe	1	-0.7	
125	EflpmKNaSfKz	1	-0.7	

2016	8	AmMaFoxEfCfCCipGmlpmKNaSSfSxCITimKzTic	1	-0.7
	16	AmAzmFoxEfCfCCipGmKNaSSfSxTeClKz	1	-0.7
	21	AmMaFoxEfCfCCipGmKNaSSfSxTeKzTic	1	-0.7
	35	AnAmMaEfCCipGmNaSSfSxTeClTic	1	-0.7
	36	AnAmEfCCipGmKNaSSfSxTeClTic	1	-0.7
	48	AmEfCfCCipGmKNaSSfSxTeClTic	1	-0.7
	65	AmEfCfCCipGmKNaSSfSxTeTic	1	-0.7
	81	AmEfCGmKNaSSfSxTeClTic	1	-0.7
	93	AmEfCGmKNaSfSxTeClTic	1	-0.7
	94	AmEfCGmKSSfSxTeClTic	1	-0.7
	96	AmEfCKNaSSfSxTeClTic	1	-0.7
	97	AmEfCNaSSfSxTeClKzTic	1	-0.7
	101	AmMaEfCfCSSfTeClTic	1	-0.7
	103	AmEfCfCGmKSSfTeTic	1	-0.7
	105	AmEfCfCSSfTeClKzTic	1	-0.7
	116	AmEfGmSSfSxTeClTic	1	-0.7
119	AmEfCSSfTeTic	1	-0.7	
124	EfCGmSSfTeCl	1	-0.7	

*Antimicrobial agents abbreviation: An: Amikacin 30ug; Am: Ampicillin 10ug; Azm: Azithromycin 15ug; Ma: Cefamandole 30ug; Fox: Cefoxitin 30ug; Ef: Ceftiofur 30ug; Cro: Ceftriaxone 30ug; Cf: Cephalothin 30ug; C: Chloramphenicol 30ug; Cip: Ciprofloxacin 15ug; Gm: Gentamicin 10ug; Ipm: imipenem 10ug; K: Kanamycin 30ug; Na: Nalidixic acid 30ug; S: Streptomycin 10ug; Sf: Sulphafurazole 300ug; Sx: Sulphamethoxazole 25ug; Te: Tetracycline 30ug; Cl: Cephalixin 30ug; Tim: Ticarcillin/Clavulanic acid 75/10ug; Va: Vancomycin 30ug; My: Lincosamin 15ug; Kz: Cephalozin 30ug; Tic: Ticarcillin 75ug

genes cause diarrhea and edema disease in post-weaning piglets. Therefore, *E. coli* infections can lead to severe economic losses in the pig industry, mostly because of medication costs, growth retardation, and mortality. Of the 139 *E. coli* isolates assessed, all isolates carried one or more of the virulence-associated genes. Among the 139 *E. coli* isolates, only 10 (7.19% of 139) had the F4ac fimbriae adhesin gene. In previous reports, the F4 *E. coli* strain was found to have high rates of prevalence, with rates of 23.4% in Canada and 44% in Denmark [28,29]. Among the 92 isolates carrying genes for fimbriae, the F18 gene variant was the most prevalent; F18 (19.5%), F18ab (50.0%), and F18ac (30.4%) were identified. In a previous report, among 63 PCR-positive isolates for 108 verotoxigenic *E. coli* and/or enterotoxigenic *E. coli*, 53 isolates (49.07%) were detected with F18ab and 10 isolates (9.26%) were detected with F18ac [10]. Among the 139 isolates carrying genes for ETEC toxins, the most prevalent toxin genes were STa (35.9%) and STb (35.2%). These results show similarity to those of a previous study, which reported STa and STb were the most frequently isolated from pigs with diarrhea [29,30]. In another previous report in Korea, Kwon, et al [31], detected the prevalence rates of F4 and F18 *E. coli* isolates, which were 4.3% and 18.3%, respectively. Additionally, the STa, STb, and Stx2e virulence genes were detected with prevalence rates of 25.7%, 15.2%, and 15.6%, respectively, in the present study. The results of both F4 and F18 fimbriae in the present study are similar to those in previous Korean studies. However, the Stx2e gene results were found to have a high prevalence rate in the *E. coli* isolates in the present study. The most common combination of genes detected for ETEC fimbriae was F18ab/LT/STa. Among the 111 isolates of ETEC, the Stx2e gene was detected in 79 (71.1%) isolates. Of these 79 isolates, F4ac (20.0%), F18 (72.2%), F18ab (67.4%), and F18ac (71.4%) carried the Stx2e gene, respectively. These results showed the combined genes for fimbriae and virulence for *E. coli* isolates were more varied and complex than previous studies have indicated.

The prevalence of antimicrobial resistance in all *E. coli* isolates

from pigs was high. All of the isolates were resistant to at least one antimicrobial, and the majority were resistant to at least seven antimicrobials. There were high rates of resistance to particular antimicrobials, notably S, Ipm, Ef, Kz, Am, Tic, Cip, Sx, and Te, regardless of the collection date. The major reason for the high resistance rates to high-level S, Ef, and Sf treatment is likely to be that these three antibiotics are commonly used as therapeutics and growth promotion agents in pig farms [16,17]. In a previous report, all *E. coli* isolates from Tibetan pigs exhibited high rates of resistance to Te, and a small number of isolates were resistant to Am, S, and Ef [32]. An Australian study reported [21] that 182 *E. coli* isolates collected from 200 pigs between 2003 and 2004 exhibited antimicrobial resistance to Am and florfenicol. A Korean study reported [33] that 744 *E. coli* isolates exhibited antimicrobial resistance to Te (96.3%), S (66.8%), Am (66.1%), and C (47.6%). Therefore, antimicrobial susceptibility results, obtained for *E. coli* from Korea and other countries, showed high levels of resistance to Te, S, and Am. The present study results were comparable to these observations, as the *E. coli* isolated from pigs showed a high prevalence of resistance to antimicrobials commonly used to treat livestock, including Te, S, Ef, and Am. Pig farms have increased the use of antimicrobials over time, which may have led to increased antimicrobial resistance among the isolates. The high prevalence of antimicrobial resistance to isolated *E. coli* strains in pig farms from Korea highlights the urgent need for measures to regulate the use of antimicrobials in pigs and other animals used for food production in Korea. By contrast, the high rates of resistance to the Ipm, Ma, and Kz of the *E. coli* isolates were decreased over the collection period. There was a high rate of susceptibility to particular antimicrobials, notably An, Cro, Va, My, and Tim, regardless of the collection date. Therefore, these antimicrobials remain potentially effective against *E. coli* species. The antimicrobial resistance patterns of 121 *E. coli* isolates were found to be AmEfCIpmKSfTeKzTic in the period between 2013 and 2015, and AmEfCfCSSfTeTic in the isolates collected in 2016; these were the most frequent resistance types. All of the AmEfCIpmKSfTeKzTic-resistant isolates collected

between 2013 and 2015, and the AmEfcCSSfTeTic-resistant isolates collected in 2016, showed high rates of resistance to the carbapenem, cepheims, penicillins, phenicols, sulfas, and tetracyclines classes and the aminoglycosides, cepheims, penicilins, phenicols, sulfas, and tetracyclines classes.

Conclusion

In the present study, the frequency of antimicrobial resistance and fimbriae-associated virulence genes were detected in *E. coli* isolates from pigs with diarrhea and/or edema disease between 2013 and 2016 in Korea. In the 139 *E. coli* isolates, variants of the fimbriae gene were detected, namely F4ac, F18, F18ab, and F18ac. Among 111 ETEC isolates, the most prevalent toxin genes, STa and STb, were detected. In addition, among these 111 ETEC isolates, 79 were detected with the presence of the Stx2 gene. Overall, these results suggest that *E. coli* disease in piglets may not be associated with a single toxin or a major gene combination, but with more varied and complex toxins and their combinations. All the *E. coli* isolates were shown to have 100% rates of resistance to Ef and Sf. By contrast, all of the isolates showed susceptibility to An, Va, My, and Tim. Our results were comparable to this observation since *E. coli* isolated from pigs showed a high prevalence of resistance to antimicrobials commonly used in livestock, including Te, S, Ef, and Am.

Acknowledgement

This research was supported by Technology Development Program for Agriculture and Forestry; the Ministry for Food, Agriculture, Forestry, and Fisheries, Republic of Korea (no. 117031-3).

References

- Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli*. *Nat Rev Microbiol*. 2004; 2: 123-140.
- Moredo FA, Piñeyro PE, Márquez GC, Sanz M, Colello R, Etcheverría A, and et al. Enterotoxigenic *Escherichia coli* Subclinical Infection in Pigs: Bacteriological and Genotypic Characterization and Antimicrobial Resistance Profiles. *Foodborne Pathog Dis*. 2015; 12: 704-711.
- Coddens A, Loos M, Vanrompay D, Remon JP, and Cox E. Cranberry extract inhibits *in vitro* adhesion of F4 and F18* *Escherichia coli* to pig intestinal epithelium and reduces *in vivo* excretion of pigs orally challenged with F18* verotoxigenic *E. coli*. *Vet Microbiol*. 2017; 202: 64-71.
- Alexa P, Stouracova K, Hamrik J, and Rychlik I. Gene typing of the colonisation factors K88 (F4) in enterotoxigenic *Escherichia coli* strains isolated from diarrhoeic piglets. *Vet Med- Czech*. 2001; 46: 46-49.
- Broeck WV, Cox E, Oudega B, and Goddeeris BM. The F4 fimbrial antigen of *Escherichia coli* and its receptor. *Vet Microbiol*. 2000; 71: 223-244.
- Holoda E, Vu-Khac H, Andrasková S, Chomová Z, Wantrubová A, Krajňák M, Pilipčinec E. PCR assay for detection and differentiation of K88ab1, K88ab2, K88ac, and K88ad fimbrial adhesins in *E. coli* strains isolated from diarrheic piglets. *Folia Microbiol*. 2005; 50: 107-112.
- Nguyen UV, Coddens A, Melkebeek V, Devriendt B, Goetstouwers T, Poucke MV, and et al. High susceptibility prevalence for F4* and F18* *Escherichia coli* in Flemish pigs. *Vet Microbiol*. 2017; 202: 52-57.
- Alexa P, Stouracova K, Hamrik J, and Salajka E. Gene typing the colonization factors F18 of *Escherichia coli* isolated from piglets suffering from post-weaning oedema disease. *Vet Med-Czech*. 2002; 47: 132-136.
- Smeds A, Pertovaara M, Timonen T, Pohjanvirta T, Pelkonen S, and Palva A. Mapping the binding domain of the F18 fimbrial adhesin. *Infect Immun*. 2003; 71: 2163-2172.
- Cheng D, Sun H, Xu J, Gao S. Prevalence of fimbrial colonization factors F18ab and F18ac in *Escherichia coli* isolates from weaned piglets with edema and/or diarrhea in China. *Vet Microbiol*. 2005; 110: 35-39.
- Chen X, Gao S, Jiao X, and Liu XF. Prevalence of serogroups and virulence factors of *Escherichia coli* strains isolated from pigs with postweaning diarrhea in eastern China. *Vet Microbiol*. 2004; 103: 13-20.
- Fairbrother JM, Nadeau E, and Gyles CL. *Escherichia coli* in postweaning diarrhea in pigs: An update on bacterial type, pathogenesis, and prevention strategies. *Anim Health Res Rev*. 2005; 6: 17-39.
- Vidotto MC, de Lima NCS, Fritzen JTT, de Freitas JC, Venancio MJ, Ono MA. Frequency of virulence genes in *Escherichia coli* strains isolated from piglets with diarrhea in the North Parana state, Brazil. *Braz J Microbiol*. 2009; 40: 199-204.
- Ha SK, Choi C, Jung K, Kim J, Han DU, Ha Y, Lee SD, Kim SH, and Chae C. Genotypic prevalence of the adhesion involved diffuse adherence in *Escherichia coli* isolates in pre-weaned pigs with diarrhea in Korea. *J Vet Med*. 2004; 51: 166-168.
- Kim YJ, Kim JH, Hur J, and Lee JH. Isolation of *Escherichia coli* from piglets in South Korea with diarrhea and characteristics of the virulence genes. *Can J Vet Res*. 2010; 74: 59-64.
- Amezcuca R, Friendship RM, Dewey CE, Gyles C, and Fairbrother JM. Presentation of postweaning *Escherichia coli* diarrhea in southern Ontario, prevalence of hemolytic *E. coli* serogroups involved, and their antimicrobial resistance patterns. *Can J Vet Res*. 2002; 66: 73-78.
- Lanz R, Kuhnert P, and Boerlin P. Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. *Vet Microbiol*. 2003; 91: 73-84.
- Agga GE, Scott HM, Amachawadi RG, Nagaraja TG, Vinasco J, Bai J, and et al. Effects of chlortetracycline and copper supplementation on antimicrobial resistance of fecal *Escherichia coli* from weaned pigs. *Prev Vet Med*. 2014; 114: 231-246.
- Luppi A, Bonilauri P, Dottori M, Gherpelli Y, Biasi G, Merialdi G, Maioli G, and Martelli P. Antimicrobial resistance of F4* *Escherichia coli* isolated from swine in Italy. *Transbound Emerg Dis*. 2015; 62: 67-71.
- Osterberg J, Wingstrand A, Jensen AN, Kerouanton A, Cibin V, Barco L, and et al. Antibiotic resistance in *Escherichia coli* from pigs in organic and conventional farming in four European countries. *PLOS one*. 2016; 11: e0157049.
- Smith MG, Jordan D, Gibson JS, Cobbold RN, Chapman TA, Abraham S, and Trott DJ. Phenotypic and genotypic profiling of antimicrobial resistance in enteric *Escherichia coli* communities isolated from finisher pigs in Australia. *Aust Vet J*. 2016; 94: 371-376.
- Gao S, Liu X, Zhang R, Jiao X, Wen Q, Wu C, and et al. The isolation and identification of pathogenic *Escherichia coli* isolates of chicken origin from some regions in China. *Acta Vet Zootech*. 1999; 30: 164-171.
- Zhang W, Zhao M, Ruescg L, Omot A, and Francis D. Prevalence of virulence factors of *Escherichia coli* strains isolated from pigs with post-weaning diarrhea. *Vet Microbiol*. 2017; 123: 145-152.
- Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Third Informational Supplement M100-S23. Wayne, PA: CLSI. 2013.
- Bertschinger HU. Postweaning *Escherichia coli* diarrhea and edema disease. In Straw BE, D'Allaire S, Mengeling WL, Taylor DJ (ed), *Diseases of swine*. Iowa State University Press, Ames, IA. 1999; 441-454.
- Mainil JG, Daube G. Verotoxigenic *Escherichia coli* from animals, humans and foods: Who's who? *J Appl Microbiol*. 2005; 98: 1332-1344.
- Osek J. Prevalence of virulence factors of *Escherichia coli* strains isolated from diarrheic and healthy piglets after weaning. *Vet Microbiol*. 1999; 68: 209-217.
- Harel J, Lapointe H, Fallara A, Lortie LA, Bigras-Poulin M, Larivière S, Fairbrother JM. Detection of genes for fimbrial antigens and enterotoxins

- associated with *Escherichia coli* serogroups isolated from pigs with diarrhea. J Clin Microbiol. 1991; 29: 745-752.
29. Frydendahl, K. 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. 2002; 85: 169-182.
30. Osek K, Trusczyński M. Occurrence of fimbriae and enterotoxins in *Escherichia coli* strains isolated from piglets in Poland. Comp Immunol Microbiol Infect Dis. 1992; 15: 285-292.
31. Kwon D, Choi C, Jung T, Chung HK, Kim JP, Bae SS, Cho WS, Kim J, Chae C. Genotypic prevalence of the fimbrial adhesins (F4, F5, F6, F41 and F18) and toxins (LT, Sta, STb, and Stx2e) in *Escherichia coli* isolated from postweaning pigs with diarrhoea or edema disease in Korea. Vet Rec. 2002; 12: 35-37.
32. Li P, Wu D, Liu K, Suolang S, He T, Liu X, Wu C, Wang Y, and Lin D. Investigation of Antimicrobial Resistance in *Escherichia coli* and Enterococci Isolated from Tibetan Pigs. PLOS one. 2014; 9: e95623.
33. Lim SK, Lee HS, Nam HM, Cho YS, Kim JM, Song SW, Park YH, and Jung SC. Antimicrobial resistance observed in *Escherichia coli* strains isolated from fecal samples of cattle and pigs in Korea during 2003-2004. Int J Food Microbiol. 2007; 116: 283-286.