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RESEARCH ARTICLE

Serogroups, Virulence Genes and Antimicrobial Resistance of F4+ and F18+ *Escherichia coli* Isolated from Weaned Piglets

Kyung-Hyo Do[1], Jae-Won Byun[2] and Wan-Kyu Lee[1*]

[1]Laboratory of Veterinary Bacteriology and Infectious Diseases, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk 28644, Republic of Korea; [2]Animal Disease Diagnostic Division, Animal and Plant Quarantine Agency, Gimcheon, Gyeongbuk 39660, Republic of Korea
*Corresponding author: wklee@cbu.ac.kr

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ABSTRACT

Ninety-one F4$^+$ pathogenic *Escherichia* (*E.*) *coli* and 181 F18$^+$ pathogenic *E. coli* were isolated from piglets suffering enteric colibacillosis during 2007-2016. These strains were analyzed for O-serogroups, adhesin genes (eae, paa, AIDA-1), toxin genes (LT, STa, STb, Stx2e, EAST-1), and their susceptibility to 16 antimicrobials using disc diffusion method. We found that O149 and O139 were predominant serogroups in F4$^+$ *E. coli* (36.3%) and F18$^+$ *E. coli* (16.6%), respectively. AIDA-1 was the most predominant adhesin gene in F18$^+$ *E. coli* (26.5%) while paa was the most predominant adhesin gene in F4$^+$ *E. coli* (30.8%). LT (70.3%), STb (84.6%), and EAST-1 (73.6%) were detected with high frequency in F4$^+$ *E. coli.* However, STa (43.6%) and Stx2e (49.2%) were the predominant toxin genes detected in F18$^+$ *E. coli*. Both F4$^+$ and F18$^+$ *E. coli* showed high resistance to tetracycline (F4$^+$: 91.2%, F18$^+$: 90.6%), chloramphenicol (F4$^+$: 87.9%, F18$^+$: 92.3%), and streptomycin (F4$^+$: 89.0%, F18$^+$: 84.0%). F18$^+$ *E. coli* showed higher resistance to colistin (9.4%) rather than F4$^+$ *E. coli* (2.2%). In summary, we compared serogroups, virulence factors, and antimicrobial susceptibility of F4$^+$ and F18$^+$ *E. coli* from diarrheic weaned piglets. Results of this study could be used to design control measures for enteric colibacillosis in piggeries.

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INTRODUCTION

Weaned piglets are vulnerable to diseases due to complex reasons such as changes in environmental conditions, decline in maternal antibody, and various stresses (Fairbrother *et al.*, 2012). Post-weaning diarrhea (PWD) and edema disease (ED) are important diseases that cause dramatic economic loss to swine farms due to diarrhea, growth retardation, and mortality (Hampson, 1994). The incidence of PWD and ED is mainly caused by virulence factors produced by pathogenic *E. coli*. Therefore, it is important to detect virulence factors of *E. coli* to diagnose colibacillosis (Duan *et al.*, 2012; Kusumoto *et al*., 2016).

Enterotoxins of pathogenic *E. coli* include heat-labile toxin (LT), heat-stable toxin (ST), enteroaggregative *E. coli* heat-stable enterotoxin 1 (EAST-1), and Shiga toxin type 2e (Stx2e) (Duan *et al.*, 2012; Kusumoto *et al.*, 2016). Among these enterotoxins, LT and ST are related to enterotoxigenic *E. coli* (ETEC) which is a well-known cause of PWD. Stx2e is related to Shiga-toxin producing *E. coli* (STEC) known to play an important role in the development of ED (Byun *et al.*, 2013).

To produce enterotoxins and cause diseases, pathogenic *E. coli* needs to attach to intestines of pigs first. Fimbriae play an important role in allowing *E. coli* to attach to the intestinal mucosa and epithelial cells. The adhesive fimbriae commonly found in pathogenic *E. coli* from piglets suffering enteric colibacillosis are F4, F5, F6, F18, and F41 (Nguyen *et al*., 2017). Especially, in weaned piglets suffering colibacillosis, the most commonly detected fimbriae are F4 and F18. Therefore, to prevent PWD and ED in piglets, strategies against F4$^+$ and F18$^+$ *E. coli* should be considered (Nguyen *et al*., 2017). The prevalence of pathogenic *E. coli* strains expressing specific fimbriae and enterotoxins is essential for controlling colibacillosis (Rhouma M *et al.,* 2017).

Antimicrobials are often used to treat colibacillosis. However, widespread and indiscriminate use of antimicrobials had led to the emergence of antimicrobial resistant bacteria, causing serious problems in treatment of disease in swine farms (Torre *et al.*, 2015). To devise control measures for colibacillosis in piggeries, data regarding the prevalence of virulence factors and antimicrobial susceptibility of *E. coli* are needed.

Information on antimicrobial resistance and distribution of pathogenic genes in F4$^+$ and F18$^+$ *E. coli* will be used useful to establish treatment and prevention strategies for colibacillosis in the swine industry. Although there have been many studies on antimicrobial resistance, and virulence characterization of pathogenic *E. coli*, studies on the comparison of virulence factor and antimicrobial resistance between *E. coli* having F4 and F18 are insufficient. In this study, we compared virulence profiles and antimicrobial resistance of *E. coli* having F4 or F18, which were the most commonly detected adherent factor.

MATERIALS AND METHODS

***E. coli* strains isolated from piglets suffering enteric colibacillosis:** From 2007 to 2016, 363 strains of *E. coli* were isolated from weaned piglets suffering enteric colibacillosis. These *E. coli* strains were isolated from 24 farms in the northern region (Gangwon, Gyeonggi, Incheon), 26 farms in the middle region (Chungbuk, Chungnam), and 50 farms in the southern region (Jeonbuk, Jeonnam, Kyungbuk, Gyeongnam). These strains were not repeatedly isolated from the same farm. The aseptically collected intestinal contents and feces were inoculated on MacConkey (BBL, USA) and blood agar (Asan Pharmaceutical, Korea). VITEK II system (bioMéreiux, France) was used to identify suspected colonies as *E. coli*. To identify F4 and F18 gene, previously described PCR protocols was used (Byun *et al.*, 2013). Of these strains, 91 strains were F4 positive, 181 strains were F18 positive, and 7 strains were both F4 and F18 positive.

O-serogroup typing: O-serogroup typing was performed using rabbit antisera purchased from SSI (Serum Staten Institute, Denmark) with slide agglutination technique of the Animal and Plant Quarantine Agency (Gimcheon, Korea). Standard strain was obtained from Dr. JM Fairbrother (*E. coli* reference laboratory, Canada).

Detection of pathogenic gene and confirmation of hemolysis: Isolated *E. coli* were cultured on a blood agar (Asan, Korea) for 18 hours at 37°C to confirm hemolytic activity. Template DNA for PCR was extracted using the boiling method (Zhang *et al.*, 2007). TaKaRa PCR Thermal Cycler Dice Gradient TP600 (Takara, Japan) was used for PCR. Enterotoxin, fimbrial and non-fimbrial adhesin genes were detected by PCR described previously (Byun *et al.*, 2013). PCR product was electrophoresed on 2% agarose gel using Mupid-exu AD140 (Takara, Japan), stained with Ethidium bromide (EtBr), and visualized on a UV transilluminator.

Antimicrobial susceptibility test: The following 16 antimicrobials were selected by referring to the Clinical and Laboratory Standards Institute (CLSI) guidance (CLSI, 2014) for this study: gentamicin (10 μg), streptomycin (10 μg), neomycin (30 μg), ampicillin (10 μg), amoxicillin / clavulanic acid (20 / 10 μg), cephalothin (30 μg), cefoxitin (30 μg), cefazolin (30 μg), cefepime (30 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), norfloxacin (10 μg), sulfamethoxazole / trimethoprim (23.75 / 1.25 μg), chloramphenicol (30 μg), colistin (10 μg), and tetracycline (30 μg). Each antimicrobial disc was purchased from Becton-Dickinson (BD, USA). Antimicrobial susceptibility testing was carried out using the Kirby Bauer disk diffusion method (Bauer *et al.*, 1966). Strains resistant to three or more CLSI subclass of drugs according to Magiorakos criteria were considered as multidrug resistant strains (Magiorakos *et al.*, 2011).

Statistical analysis: All statistical analyses were performed using SPSS version 12.0 program (SPSS, Chicago, IL, USA). Chi-square test was performed to analyze pathogenic characteristics and antimicrobial resistance rate of F4$^+$ and F18$^+$ *E. coli*.

RESULTS

O-serogroups and Hemolysis of F4$^+$ and F18$^+$ *E. coli:* Results of O-serogroups of F4$^+$ and F18$^+$ *E. coli* are shown in Table 1. While 33 (36.3%) strains among 91 strains of F4$^+$ *E. coli* were O149, 30 (16.6%) strains among 165 strains of F18$^+$ *E. coli* were O139. In the O-rough group, only one (1.1%) strain was F4$^+$ while 22 (12.2%) strains were F18$^+$. Regarding non-typeable serotype which was not detected in standard O-antiserum, 7 (7.7%) strains were detected to be F4$^+$ *E. coli* while 43 (23.8%) strains were detected to be F18$^+$, showing significantly higher detection rates. In terms of hemolysis, regardless of fimbrial adhesin gene, 87.9% (80 of 91 strains) of F4$^+$ *E. coli* and 91.2% (165 of 181 strains) of F18$^+$ *E. coli* were highly hemolytic.

Prevalence of Non-fimbrial adhesin and Toxin genes of F4$^+$ and F18$^+$ *E. coli:* Non-fimbrial adhesin and various enterotoxin genes were tested for F4$^+$ and F18$^+$ *E. coli* (Table 2). 26.5% (48 of 181 strains) of F18$^+$ *E. coli* were AIDA-1 positive, showing that AIDA-1 was the most prevalent non-fimbrial adhesin factor. Of 91 strains of F4$^+$ *E. coli*, 28 (30.8%) were paa positive, and also 42 (23.2%) strains of F18$^+$ *E. coli* were paa positive showing high detection rates of paa genes in both F4$^+$ and F18$^+$ *E. coli*. Only one (4.4%) strain was AIDA-1 positive in F4$^+$ *E. coli*. Eae gene was detected in 2 (2.2%) of 91 strains of F4$^+$ *E. coli* and 3 (1.7%) of 181 strains of F18$^+$*E. coli*.

Table 1: O-serogroups and hemolysis pattern of *E. coli* encoding F4 or F18 gene isolated from diarrheic weaned piglets in Korea from 2007 to 2016

O-serogroup	F4		F18	
	No. (%)	Hemolysis	No. (%)	Hemolysis
O149$^{**\ 1)}$	33 (36.3)$^{2))}$	31(93.9)$^{3)0}$	4 (2.2)	3 (75.0)
O139**	2 (2.2) $^{1)}$	1 (50.0) $^{1)}$	30 (16.6)	25 (83.3)
O157	7 (7.7) $^{1)}$	7 (100.0) $^{1)}$	5 (2.8)	5 (100.0)
Others $^{4)}$	41 (45.1) $^{1)}$	34 (82.9) $^{1)}$	77 (42.5)	73 (94.8)
OR $^{5)\ **}$	1 (1.1) $^{1)}$	1 (100.0) $^{1)}$	22 (12.2)	21 (95.5)
NT $^{6)\ **}$	7 (7.7) $^{1)}$	6 (85.7) $^{1)}$	43 (23.8)	38 (88.4)
Total	91 (100.0) $^{1)}$	80 (87.9) $^{1)}$	181 (100.0)	165 (91.2)

$^{1)}$ Significant difference between F4$^+$and F18$^+$ *E. coli* (P<0.01). $^{2)}$ No. of O-serogroup isolates / No. of F4$^+$ or F18$^+$ *Escherichia coli* isolates × 100 (%) $^{3)}$ No. of hemolytic O-serogroup isolates / No. of O-serogroup isolates × 100 (%) $^{4)}$ Other serogroup: O2, O7, O8, O9, O10, O11, O14, O20, O24, O28, O35, O39, O50, O71, O73, O76, O86, O98, O100, O107, O109, O111, O117, O120, O121, O127, O136, O141, O146, O153, O154, O182 $^{5)}$ O-rough: non-specific reaction $^{6)}$ Untypeable.

Table 2: Frequency of non-fimbrial adhesins and toxigenic genes among *E. coli* encoding F4 or F18 gene isolated from diarrheic weaned piglets in Korea from 2007 to 2016

Non-fimbrial adhesins and Toxins		F4 (n=91)	F18 (n=181)
Non-fimbrial adhesins	AIDA-I** [1)]	01 (4.4) [2)]	48 (26.5)
	paa	28 (30.8) [2)]	42 (23.2)
	eae	02 (2.2) [2)]	0 3 (1.7)
Toxins	LT**	64 (70.3) [2)]	72 (39.8)
	STa**	24 (26.4) [2)]	79 (43.6)
	STb**	77 (84.6) [2)]	38 (21.0)
	EAST-1**	67 (73.6) [2)]	65 (35.9)
	Stx2e**	08 (8.8) [2)]	89 (49.2)

[1)] Significant difference between F4$^+$ and F18$^+$ *E. coli* (P<0.01). Data were expressed as No. (%) of isolates.

Table 3: Antimicrobial resistance of *E. coli* encoding F4 or F18 gene isolated from diarrheic weaned piglets in Korea from 2007 to 2016

Antimicrobial subclass	Antimicrobial agents	No. of resistant isolates (Antimicrobial resistance %)	
		F4 (n=91)	F18 (n=181)
Aminoglycosides	Gentamicin* [1)]	69 (75.8)00	109 (60.2)00
	Streptomycin	81 (89.0)00	152 (84.0)00
	Neomycin	70 (76.9)00	119 (65.7)00
1st generation cephalosporin	Cephalothin**	44 (48.4)00	120 (66.3)00
	Cefazolin**	14 (15.4)00	63 (34.8)00
4th generation cephalosporin	Cefepime	2 (2.2)00	2 (1.1)00
Cephamycin	Cefoxitin**	7 (7.7)00	48 (26.5)00
Quinolones	Nalidixic acid**	83 (91.2)00	141 (77.9)00
Fluoroquinolone	Ciprofloxacin	55 (60.4)00	109 (60.2)00
	Norfloxacin	51 (56.0)00	103 (56.9)00
Aminopenicillin	Ampicillin	77 (84.6)00	153 (84.5)00
β-Lactam / β-lactamase-inhibitor combination	Amoxicillin / Clavulanic acid*	29 (31.9)00	86 (47.5)00
Folate-pathway inhibitors	Trimethoprim / Sulfamethoxazole	55 (60.4)00	130 (71.8)00
Phenicols	Chloramphenicol	80 (87.9)00	167 (92.3)00
Polymyxins	Colistin*	2 (2.2)00	17 (9.4)00
Tetracyclines	Tetracycline	83 91.2)00	164 (90.6)00

[1)] Significant differences between F4$^+$ and F18$^+$ *E. coli* were expressed as * (P<0.05) and ** (P<0.01).

Table 4: Multi-drug resistance pattern of *E. coli* encoding F4 or F18 gene isolated from diarrheic weaned piglets in Korea from 2007 to 2016

No. of resistance[1)]	No. of resistant isolates (Antimicrobial resistance %)	
	F4 (n=91)	F18 (n=181)
0 subclass	0 (0.0)000000	4 (2.2)000000
1 subclass	0 (0.0)000000	3 (1.7)000000
2 subclasses	0 (0.0)000000	3 (1.7)000000
3 subclasses	4 (4.4)000000	5 (2.8)000000
4 subclasses	6 (6.6)000000	5 (2.8)000000
5 subclasses	12 (13.2)000000	20 (11.0)000000
6 subclasses	14 (15.4)000000	23 (12.7)000000
7 subclasses** [2)]	30 (33.0)000000	31 (17.1)000000
8 subclasses	12 (13.2)000000	24 (13.3)000000
9 subclasses	8 (8.8)000000	19 (10.5)000000
10 subclasses**	5 (5.5)000000	41 (22.7)000000
11 subclasses	0 (0.0)000000	3 (1.7)000000
12 subclasses	0 (0.0)000000	0 (0.0)000000
Multi-resistant* (≥ 3 subclasses)	91 (100.0)000000	171 (94.5)000000

[1)]Antimicrobial subclasses defined by the Clinical and Laboratory Standards Institute (CLSI) are used. [2)]Significant differences between F4$^+$ and F18$^+$ *E. coli* were expressed as * (P<0.05) and ** (P<0.01).

Though detection rates of LT, STb and EAST-1 toxin gene in F4$^+$ *E. coli* were 70.3, 84.6 and 73.6%, which were high, the rates were 39.8%, 21.0%, and 35.9% in F18$^+$ *E. coli*, respectively, lower than half of that in F4$^+$ *E. coli*. However, regarding STa and Stx2e genes, detection rates in F18$^+$ *E. coli* were 43.6% and 49.2%, showing significantly high rates compared to rates in F4$^+$ *E. coli* (26.4% and 8.8%, respectively).

Comparison of Antimicrobial resistance rates between F4$^+$ and F18$^+$ *E. coli:* Results of antimicrobial resistance of *E. coli* with F4 and F18 genes are shown in Table 3. Both showed high resistance to tetracycline (F4$^+$: 91.2%, F18$^+$: 90.6%), chloramphenicol (F4$^+$: 87.9%, F18$^+$: 92.3%), streptomycin (F4$^+$: 89.0%, F18$^+$: 84.0%), ampicillin (F4$^+$: 84.6%, F18$^+$: 84.5%), and nalidixic acid (F4$^+$: 91.2%, F18$^+$: 77.9%). However, some strains showed low resistance to cefepime (F4$^+$: 2.2%, F18$^+$: 1.1%) and colistin (F4$^+$: 2.2%, F18$^+$: 9.4%).

When antimicrobial resistance rates of F4$^+$ and F18$^+$ *E. coli* were compared, resistance rates of F4$^+$ *E. coli* to gentamicin (F4$^+$: 75.8%, F18$^+$: 60.2%) and nalidixic acid (F4$^+$: 91.2%, F18$^+$: 77.9%) were significantly higher than those of F18$^+$ *E. coli*. On the other hand, resistance rates of F18$^+$ *E. coli* to cephalothin (F4$^+$: 48.4%, F18$^+$: 66.3%), cefazolin (F4$^+$: 15.4%, F18$^+$: 34.8%), cefoxitin (F4$^+$: 7.7%, F18$^+$: 26.5%), amoxicillin/clavulanic acid (F4$^+$: 31.9%, F18$^+$: 47.5%), and colistin (F4$^+$: 2.2%, F18$^+$: 9.4%) were significantly higher than those of F4$^+$ *E. coli.*

Multidrug resistance rates of F4$^+$ and F18$^+$ *E. coli:* Results of analysis of multidrug resistance rates of F4$^+$ and F18$^+$ *E. coli* are shown in Table 4. For F4$^+$ *E. coli*, 33.0% showed pattern of resistance to 7 subclasses. This multidrug resistant rate was significantly higher than that (17.1%) of F18$^+$ *E. coli*. For F18$^+$ *E. coli*, 22.7% showed pattern of resistance to 10 subclasses, which was significantly higher than that (5.5%) of F4$^+$ *E. coli*

In terms of multidrug resistance for those having resistance to 3 or more subclasses of drugs among 12 subclasses of drugs tested, 91 (100%) strains of F4$^+$ *E. coli* and 171 (94.5%) out of 181 stains of F18$^+$ *E. coli* showed multidrug resistance.

DISCUSSION

Due to *E. coli* infection, domestic swine farms are suffering from high mortality and growth retardation, causing dramatic economic loss. To cause colibacillosis, pathogenic *E. coli* must first adhere to the intestinal mucosa of piglets. Pathogenic *E. coli* then proliferates to produce enterotoxin which causes clinical symptoms such as diarrhea. Thus, adhesin factors such as fimbriae may play an important role in the pathogenesis of colibacillosis (Melkebeek *et al.*, 2013; Nguyen *et al.*, 2017). In this study, we investigated virulence factors and antimicrobial resistance of F4$^+$ and F18$^+$ *E. coli* among *E. coli* isolated from weaned piglets suffering enteric colibacillosis.

In this study, F18$^+$ *E. coli* (181 strains) detected about twice as many as F4$^+$ *E. coli* (91 strains). Fimbriae can bind to specific receptors on the surface of the intestinal mucosa. While F4 receptors are predominant in suckling piglets, the number of F18-receptors begin to increase gradually with age (Fairbrother *et al.*, 2012). Due to this change, F18$^+$ *E. coli* was detected more than F4$^+$ *E. coli* in the present study.

There are various serogroups of *E. coli*. However, only some serotypes are associated with porcine intestinal disease. The frequency of detection is known to vary depending on the region and time. Regional differences and other selective benefits are known to be involved in the survival of particular serotypes in porcine intestinal environment (Vila *et al.*, 2016).

Kwon *et al.* (1999) have reported that O157 and O8 are the most prevalent serotypes in Korea. However, major serotypes detected in this study were O149 ($F4^+$: 36.3%) and O139 ($F18^+$: 16.6%). O157 was detected in only 7 (7.7%) $F4^+$ *E. coli* strains and 5 (2.8%) $F18^+$ *E. coli* strains. Therefore, although O157 is still present in domestic piglets, O149 and O139 are becoming the most prevalent serotypes. O149 is known to be the serotype associated with ETEC. It is commonly found in pigs with PWD. O139 is a serotype associated with STEC. It is frequently detected in piglets with ED (Fairbrother *et al.*, 2012, Vila *et al.*, 2016). Kusumoto *et al.* (2016) have reported that O149 is associated with F4 while O139 is associated with F18. The same result was found in this study. As shown in Table 1, O149 was detected in 36.3% of $F18^+$ *E. coli* and 2.2% of $F4^+$ *E. coli* while O139 was detected in 16.6% of $F18^+$ *E. coli*.

Hemolysin is known to be one of the pathogenic factors of *E. coli* (Fairbrother *et al.*, 2012). $F4^+$ *E. coli* is characterized by its hemolysin production capability *in vitro* (Delannoy *et al.*, 2017). Kim *et al.* (2010) have shown the association of hemolytic *E. coli* isolated from diarrhea piglets with fimbrial adhesin genes such as F5 and F18. In the present study, hemolytic activity was seen in 87.9% of $F4^+$ *E. coli* and 91.2% of $F18^+$ *E. coli*, confirming that $F4^+$ and $F18^+$ *E. coli* were highly related to hemolysis.

AIDA-1 is associated with EAST-1 and ST genes and usually detected in $F18^+$ *E. coli* (Duan *et al.*, 2017). In the present study, detection frequency of AIDA-1 was 26.5% in $F18^+$ *E. coli*, which was significantly higher than that (4.4%) in $F4^+$ *E. coli*. An *et al.* (1999), Leclerc *et al.* (2007), Baranzoni *et al.* (2016), and Delannoy *et al.* (2017) have reported that paa-positive strains have higher association with F4 than with F18. However, in the present study, paa was detected at high frequency in both $F4^+$ *E. coli* (30.8%) and $F18^+$ *E. coli* (23.2%). There was no significant difference in the detection ratio between $F4^+$ and $F18^+$ *E. coli*. Although it is currently unclear what role paa specifically plays in the expression of the disease, genes known to be detected in $F4^+$ *E. coli* are also frequently detected in $F18^+$ *E. coli* (Fairbrother *et al.*, 2005; Nguyen *et al*., 2017).

As a result of examining antimicrobial resistance rates of $F4^+$ and $F18^+$ *E. coli* (Table 3), both were highly resistant to tetracycline, chloramphenicol, streptomycin, and ampicillin. This is similar to the monitoring results done in Denmark (DANMAP, 2017), Canada (Government of Canada, 2016), and Japan (JVARM, 2016). In the study on susceptibility test of *E. coli* isolated from pigs by Lim *et al* (2014), rates of resistance to tetracycline, ampicillin, and streptomycin were 76.1%, 64.6% and 58.4%, respectively. In a recently published study by Park *et al.* (2016), similar result was reported. Rates of resistance to tetracycline, ampicillin were 87.5%, 93.8%, respectively, showing the highest resistance rates among tested antimicrobial agents.

As a result of comparison of antimicrobial resistance rates of $F4^+$ and $F18^+$ *E. coli*, $F4^+$ *E. coli* showed significantly higher rates of resistance to gentamicin and nalidixic acid than $F18^+$ *E. coli* while $F18^+$ *E. coli* showed significantly higher rates of resistance to cephalothin, cefazolin, cefoxitin, amoxicillin / clavulanic acid and colistin than $F4^+$ *E. coli*. This might be due to differences in administered antimicrobials according to age. $F4^+$ *E. coli* is predominant in suckling piglet while $F18^+$ *E. coli* is predominantly detected as age increases (Fairbrother *et al.*, 2005; Vila *et al*., 2016). Aminoglycosides (such as gentamicin, streptomycin, and neomycin) and quinolones (such as nalidixic acid) are commonly used for prevention and treatment of diarrhea in suckling piglets (Fairbrother *et al.*, 2012). Since these antimicrobials were administered when piglets were at their suckling period, antimicrobial resistance rates of $F4^+$ *E. coli* present in suckling piglets were higher than those of $F18^+$ *E. coli*. On the other hand, cephalosporin class antimicrobials are second and last-choice drugs that are used only when first-choice antimicrobials fail to work. They are used more in weaned piglets than in suckling piglets. Different types of antimicrobials used for pigs depending on their age might have caused difference in resistance rates between $F4^+$ and $F18^+$ *E. coli*.

The present study showed that the frequency of multidrug-resistant bacteria that were resistant to more than three antimicrobial subclasses was very high ($F4^+$: 100%, $F18^+$: 94.5%). Our results showed much higher multidrug resistance rates compared to those (38.7%) reported in Italy diseased pigs-derived *E. coli* (Luppi *et al.*, 2015), although it was difficult to directly compare these rates between studies since different antimicrobials were used. Given that regulations on the use of antimicrobials in Korea are not as strict as those in developed countries, wide use of antimicrobials by non-experts such as livestock-related workers rather than veterinarians might be the reason for such high resistance rates in Korea (Cho *et al.*, 2006). This study provides useful information on antimicrobial resistance and distribution of pathogenic genes in $F4^+$ and $F18^+$ *E. coli* isolated from weaned piglets suffering enteric colibacillosis. Our findings provide important information on antimicrobial resistance to veterinarians, but also could be used to establish treatment and prevention strategies for colibacillosis in the swine industry. Further studies are needed to determine the specific association of virulence factors and antimicrobial resistance with fimbrial gene.

Conclusions: This study analyzed the virulence factors and antimicrobial resistance of *E. coli* carrying F4 or F18 fimbria. In $F4^+$ *E. coli*, O149 (36.3%) and EAST-1 (73.6%) were detected significantly higher, nalidixic acid (91.2%) showed higher resistance than $F18^+$ *E. coli*. Meanwhile, O139 (16.6%), AIDA-1 (26.5%) and Stx2e (49.2%) were detected higher in $F18^+$ *E. coli*. And also, $F18^+$ *E. coli* showed higher resistance in cephalothin (66.3%), cefazolin (34.8%), cefoxitin (26.5%) and colistin (9.4%) than $F4^+$ *E. coli*. Our findings showed there were differences in virulence factors and antimicrobial resistance between $F4^+$ and $F18^+$ *E. coli*.

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Authors contribution: KD, JW, and WL conceived and planned the study. KD, JB and WL performed the analysis, drafted manuscript. KD and JB carried out the experiment. KD wrote the manuscript in consultation with JB and WL.

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