



RESEARCH ARTICLE

Antimicrobial Resistance, Adhesin and Toxin Genes of Porcine Pathogenic *Escherichia coli* Following the Ban on Antibiotics as the Growth Promoters in Feed

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ABSTRACT

To prevent and treat colibacillosis efficiently, a thorough understanding of the antimicrobial and virulence profiles present in porcine pathogenic *Escherichia* (*E.*) *coli* is needed. In this study, we isolated 196 pathogenic *E. coli* from the grower and finisher pigs with diarrhea for six years after the ban (July 2011) on antibiotics as the growth promoters in feed (2011–2016) in Korea. And we tested antimicrobial susceptibility and determined the prevalence of adhesin and toxin genes in these isolates. Based on the Clinical and Laboratory Standards Institute guidelines, we surveyed antimicrobial resistance of the pathogenic *E. coli* isolates. The most prevalent adhesin gene was F18 (43.9%), followed by AIDA-1 (37.4%). After 2013, the frequency of *paa*, which plays a role in attaching and effacing adhesion, decreased from 16.8% to 2.2%. The most prevalent pathotype was enterotoxigenic *E. coli* (49.5%), and its frequency increased from 43.0% to 57.3% after 2013. We confirmed high resistance rates to cephalothin (96.4%), ampicillin (93.9%), tetracycline (87.2%), and chloramphenicol (85.7%). After 2013, a decrease in gentamicin resistance was observed (from 52.3% to 10.1%). However, resistance to almost all other antimicrobials tested increased, especially for cefazolin (42.1% to 76.4%), cefepime (7.5% to 16.9%), cefoxitin (12.1% to 20.2%), and colistin (32.7% to 62.9%). Most isolates (98.0%) exhibited multidrug resistance. The results of this study could be used for the efficient development of control measures for enteric colibacillosis in piggeries.

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INTRODUCTION

Escherichia (*E.*) *coli* is a common causative agent of diseases in pigs, other livestock species, and humans. In pigs, *E. coli* infections are mainly associated with enteritis, edema disease, and septicemia. Pigs are considered the primary reservoirs of pathogenic *E. coli* in human infections and food products, such as pork products, and the vehicles for the transmission of pathogenic *E. coli* (Fairbrother *et al.*, 2012). *E. coli* is one of the most common enterobacteria and can serve as a reservoir for antimicrobial resistance genes.

Shiga toxin-producing *E. coli* (STEC) strains encoding the Stx2e genes can cause edema disease in pigs, specifically postweaning and young finishing pigs.

Enterotoxigenic *E. coli* could produce the heat-labile (LT) and heat-stable (ST; STa, STb, EAST-I) toxin, which are the causes of diarrhea. (Byun *et al.*, 2013).

After attaching to the intestinal mucosa and epithelial cells; pathogenic *E. coli* produce enterotoxins. And in this process, adhesins play a significant role (Duan *et al.*, 2012). To diagnose the enteric colibacillosis, the identification of these virulence factors is the key. Because the virulence factors determine the pathogenicity of *E. coli*. (Fairbrother *et al.*, 2012).

To control the diseases in pigs, antibiotics play a significant role. For many years, antibiotics were frequently used in the swine industry as the form of feed additives to prevent diseases and promote growth in Korea (Do *et al.*, 2020a). However, antimicrobial-resistant

bacteria and antibiotic residues in meat products could appear due to the indiscriminate use of antimicrobial agents. As such, antibiotics for the growth promoters (AGPs) were entirely banned in Korea in July 2011 (Animal and Plant Quarantine Agency, 2019).

Antimicrobial resistance has emerged as a global health problem owing to the widespread use and misuse of antibiotics. To overcome this problem, many developed countries including Denmark (DANMAP, 2013), Japan (JVARM, 2016) and Canada (the Government of Canada, 2014) monitor the antimicrobial use and resistance formally. The antimicrobial resistance, and virulence profiles of *E. coli* are variable depending on the region and time (Do *et al.*, 2019). Numerous studies have been surveyed the antimicrobial resistance and virulence factors of *E. coli* strains isolates from pigs in Korea, but there is little available data concerning the age group of livestock, especially for grower and finisher pigs, which are of particular importance due to their close association with humans (Lim *et al.*, 2014). Although, after the ban on AGPs, data about changes in antimicrobial resistance are important to design efficient treatment and prevention strategies against colibacillosis, there is little data on antimicrobial resistance patterns of *E. coli* isolated from grower and finisher pigs. We isolated 196 *E. coli* isolates after the ban on AGPs (2011-2016) from Korean diarrheic grower and finisher pigs.

In the present study, we surveyed antimicrobial resistance and virulence factors of *E. coli* isolates during the six years after the ban on AGPs (2011-2016).

MATERIALS AND METHODS

***E. coli* strains:** Between 2011 and 2016, 196 *E. coli* isolates were obtained from piglets that exhibited symptoms of diarrhea or edema disease. The sampled farms consisted of 98 different pig herds (50 to 100 sows per herd) and were located in three areas: northern (21 farms in the Gangwon, Gyeonggi, and Incheon provinces), central (44 farms in the Chungbuk, Chungnam, and Gyeongbuk provinces), and southern (33 farms in the Chonbuk, Chonnam, and Gyeongnam provinces) South Korea (Figure 1). Strains were not collected repeatedly from the same farm. The aseptically collected intestinal and fecal samples were inoculated onto MacConkey agar (Becton Dickinson, MD, USA). After overnight incubation at 37 °C, pure, pink-colored colonies were selected and transferred onto blood agar (Asan Pharmaceutical, Korea). Suspected colonies were identified as *E. coli* using the VITEK II system (bioMérieux, Marcy l'Etoile, France). The tested isolates were stored in 50% glycerol stock at -70 °C until further characterization.

Determination of virulence genes: The *E. coli* genes for the toxins (LT, STa, STb, Stx2e, and EAST-1), fimbrial adhesins (F4, F5, F6, F18, and F41), and non-fimbrial adhesins (AIDA-1, paa, and eae) were amplified by polymerase chain reaction (PCR) following previously described protocols (Do *et al.*, 2019a). Bacterial colonies were suspended in distilled water and boiled for 10 min. After centrifugation at 8,000 × g, the supernatant was used as a template for PCR. The reaction volume (20 µL)

was composed of 2 × EmeraldAmp Master Mix (Takara, Japan), 2 µM of each primer, and 3 µL of DNA template. After amplification, the products were visualized by electrophoresis on 2% agarose gels stained with ethidium bromide.

Antimicrobial resistance: Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method (Bauer *et al.*, 1966). The following 16 antimicrobials were selected following the marketing amounts for animal use in Korea (Animal and Plant Quarantine Agency, 2019) and after referring to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical & Laboratory Standards Institute, 2018): gentamicin (10 µg), streptomycin (10 µg), neomycin (30 µg), cephalothin (30 µg), cefazolin (30 µg), cefepime (30 µg), ceftiofur (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), trimethoprim/sulfamethoxazole (23.75/1.25 µg), chloramphenicol (30 µg), colistin (10 µg), and tetracycline (30 µg). Antimicrobial discs were purchased from Becton Dickinson (BD, USA). Strains resistant to three or more CLSI subclasses of drugs according to the Magiorakos criteria were considered as multi-drug resistant strains (Magiorakos *et al.*, 2011).

RESULTS

Colonization factors and Toxin genes: We described the frequencies of the fimbrial adhesin, non-fimbrial adhesin, and toxin genes of pathogenic *E. coli* strains in Table 1. F18 (78, 39.8%) was the most prevalent fimbrial antigen, followed by F4 (18, 9.2%). In contrast, only four (2.0%) isolates were found to be F5- and F41-positive, and only one (0.5%) isolate was found to be F6-positive. AIDA-I was the most frequently detected non-fimbrial adhesins (70, 35.7%). Additionally, no isolates encoding the *eae* gene was detected. Between 2011 and 2013, the *paa* gene was detected in 16.8% (18 out of 107) of samples. However, between 2014 and 2016, only two (2.2%) isolates tested positive for *paa*.

Stx2e (88, 44.8%) was the most prevalent toxin genes, STb (86, 43.9%), and EAST-I (85, 43.4%). The frequency of toxin genes was slightly changed over time. The most prevalent toxin gene from 2011 to 2013 was Stx2e (60 out of 107, 56.1%). However, between 2014 and 2016, STb was the most frequently detected toxin gene (37 out of 89, 41.6%).

Pathotypes and virotypes (combination of colonization factors and toxin genes): The combination of colonization factors and toxin genes (virotypes) is presented in Table 2. The most prevalent pathotype was ETEC (49.5%), followed by STEC (27.6%). Before 2011, the frequency of ETEC was 43.0%; however, it increased to 57.3% between 2014 and 2016. Otherwise, detection ratio of STEC decreased to 21.3% from 32.7%. The most prevalent virotype was AIDA:STb:EAST-I (ETEC), followed by F18:AIDA:Stx2e (STEC). From 2011 to 2013, only one isolate was identified as virotype STa:STb. However, between 2014 and 2016, seven isolates were identified.

Table 1: Colonization factors and toxin genes of *Escherichia coli* from diarrheic pigs isolated during 6 years after the ban on antibiotic growth promoters in feed (2011–2016)

Colonization factor		2011-2013 (n = 107)	2014-2016 (n = 89)	2011-2016 (n = 196)
Fimbriae	F4	10 (9.3%)	8 (9.0%)	18 (9.2%)
	F5	2 (1.9%)	2 (2.2%)	4 (2.0%)
	F6	0 (0.0%)	1 (1.1%)	1 (0.5%)
	F18	47 (43.9%)	31 (34.8%)	78 (39.8%)
	F41	1 (0.9%)	3 (3.4%)	4 (2.0%)
Non-fimbrial adhesins	eae	0 (0.0%)	0 (0.0%)	0 (0.0%)
	paa	18 (16.8%)	2 (2.2%)	20 (10.2%)
	AIDA-I	40 (37.4%)	30 (33.7%)	70 (35.7%)
Toxins	LT	24 (22.4%)	14 (15.7%)	38 (19.4%)
	STa	31 (29.0%)	27 (30.3%)	58 (29.6%)
	STb	49 (45.8%)	37 (41.6%)	86 (43.9%)
	Stx2e	60 (56.1%)	28 (31.5%)	88 (44.9%)
	EAST-I	50 (46.7%)	35 (39.3%)	85 (43.4%)

Data are expressed as number (%) of isolates.

Table 2: Pathotypes and virotypes of *Escherichia coli* from diarrheic pigs isolated during 6 years after the ban on antibiotic growth promoters in feed (2011–2016)

Pathotypes	2011-2013 (n = 107)	2014-2016 (n = 89)	2011-2016 (n = 196)
ETEC	46 (43.0%)	51 (57.3%)	97 (49.5%)
AIDA:STb:EAST I	15	8	23
STa:STb	1	7	8
F4:LT:STb:EAST I	4	3	7
F18:STa	2	3	5
AIDA:LT:STb:EAST I	1	4	5
Others	23	26	49
STEC	35 (32.7%)	19 (21.3%)	54 (27.6%)
F18:AIDA:Stx2e	14	7	21
Stx2e	7	6	13
F18:Stx2e:EAST I	4	2	6
F18:Stx2e	1	2	3
Stx2e:EAST I	3	0	3
Others	6	2	8
ETEC/STEC	25 (23.4%)	9 (10.1%)	34 (17.3%)
STa:STb:Stx2e	3	2	5
F18:LT:Stx2e	4	1	5
F18:LT:STa:Stx2e	3	1	4
Others	15	5	20
None	1 (0.9%)	10 (11.2%)	11 (5.6%)

Data are expressed as number (%) of isolates.

Antimicrobial resistance: Table 3 describes the results of the antimicrobial susceptibility test. High resistance to cephalothin (189 isolates, 96.4%), ampicillin (184 isolates, 93.9%), tetracycline (171 isolates, 87.2%), and chloramphenicol (168 isolates, 85.7%) was confirmed in this study. In contrast, the isolates showed low resistance to cefepime (23 isolates, 11.7%) and ceftiofur (31 isolates, 15.8%). The isolates also exhibited moderate antimicrobial resistance to ceftazidime (113 isolates, 57.7%) and colistin (91 isolates, 46.4%).

A difference was observed in the aminoglycosides resistance profiles. Isolates were found to have higher resistance to streptomycin (159 isolates, 81.1%) and neomycin (156 isolates, 79.6%) than gentamicin (65 isolates, 33.2%). Resistance to gentamicin became lower during 2014–2016 (9 isolates, 10.1%), compared to that in 2011 to 2013 (56 isolates, 52.3%). However, the resistance rates for most of the antimicrobial agents tested have increased, such as in the case of ceftazidime (42.1% to 76.4%), cefepime (7.5% to 16.9%), ceftiofur (12.1% to 20.2%), ciprofloxacin (33.6% to 52.8%), norfloxacin (27.1% to 41.6%), and colistin (32.7% to 62.9%).

Multidrug resistance: Table 4 shows the multiple resistances of pathogenic *E. coli* isolates. All isolates were

Table 3: Antimicrobial resistance of *Escherichia coli* from diarrheic pigs isolated during 6 years after the ban on antibiotic growth promoters in feed (2011–2016)

Antimicrobial subclass	Antimicrobial agents	2011-2013 (n = 107)	2014-2016 (n = 89)	2011-2016 (n = 196)
Aminoglycosides	Gentamicin	56 (52.3%)	9 (10.1%)	65 (33.2%)
	Streptomycin	79 (73.8%)	80 (89.9%)	159 (81.1%)
	Neomycin	80 (74.8%)	76 (85.4%)	156 (79.6%)
Cephalosporin I	Cephalothin	101 (94.4%)	88 (98.9%)	189 (96.4%)
	Cefazolin	45 (42.1%)	68 (76.4%)	113 (57.7%)
Cephalosporin IV	Cefepime	8 (7.5%)	15 (16.9%)	23 (11.7%)
	Ceftiofur	13 (12.1%)	18 (20.2%)	31 (15.8%)
Quinolones	Nalidixic acid	67 (62.6%)	64 (71.9%)	131 (66.8%)
Fluoroquinolone	Ciprofloxacin	36 (33.6%)	47 (52.8%)	83 (42.3%)
	Norfloxacin	29 (27.1%)	37 (41.6%)	66 (33.7%)
Aminopenicillin β-lactam / β-lactamase inhibitor combination	Ampicillin	99 (92.5%)	85 (95.5%)	184 (93.9%)
	AMC ^{a)}	76 (71.0%)	78 (87.6%)	154 (78.6%)
Folate-pathway inhibitors	SXT ^{b)}	69 (64.5%)	67 (75.3%)	136 (69.4%)
Phenicol	Chloramphenicol	92 (86.0%)	76 (85.4%)	168 (85.7%)
	Colistin	35 (32.7%)	56 (62.9%)	91 (46.4%)
Tetracyclines	Tetracycline	92 (86.0%)	79 (88.8%)	171 (87.2%)

Data are expressed as number (%) of isolates. ^{a)} Amoxicillin / Clavulanic acid. ^{b)} Trimethoprim / Sulfamethoxazole.

Table 4: Multiple resistance of 196 *Escherichia coli* from diarrheic pigs in Korea isolated during 6 years after the ban on antibiotic growth promoters in feed (2011–2016)

No. of resistance ^{a)}	2011-2013 (n = 107)	2014-2016 (n = 89)	2011-2016 (n = 196)
0 subclass	0 (0.0%)	0 (0.0%)	0 (0.0%)
1 subclass	2 (1.9%)	0 (0.0%)	2 (1.0%)
2 subclasses	2 (1.9%)	0 (0.0%)	2 (1.0%)
3 subclasses	2 (1.9%)	0 (0.0%)	2 (1.0%)
4 subclasses	4 (3.7%)	1 (1.1%)	5 (2.6%)
5 subclasses	7 (6.5%)	4 (4.5%)	11 (5.6%)
6 subclasses	15 (14.0%)	7 (7.9%)	22 (11.2%)
7 subclasses	20 (18.7%)	13 (14.6%)	33 (16.8%)
8 subclasses	15 (14.0%)	17 (19.1%)	32 (16.3%)
9 subclasses	25 (23.4%)	16 (18.0%)	41 (20.9%)
10 subclasses	11 (10.3%)	18 (20.2%)	29 (14.8%)
11 subclasses	4 (3.7%)	9 (10.1%)	13 (6.6%)
12 subclasses	0 (0.0%)	4 (4.5%)	4 (2.0%)
Multi-resistant (≥ 3 subclasses)	103 (96.3%)	89 (100.0%)	192 (98.0%)

Data are expressed as number (%) of isolates. ^{a)} Antimicrobial subclasses defined by the Clinical and Laboratory Standards Institute are used.

resistant to at least one antimicrobial agent. Most isolates exhibited multidrug resistance (192 isolates, 98.0%). Between 2011 and 2013, 103 isolates (96.3%) were found to be resistant to multiple drugs. However, between 2014 and 2016, all isolates (100%) were found to be multi-drug resistant. Additionally, four isolates (4.5%) were resistant to 12 antimicrobial subclasses during 2014–2016.

DISCUSSION

In the swine industry, enteric colibacillosis is the critical disease due to the significant economic losses worldwide. Therefore, it is very important to prevent this disease by establishing measures, such as the efficient use of medicines or vaccine development. To develop an efficient vaccine, information about the distribution of adhesin and toxin genes is essentially needed. To this end, the antimicrobial resistance profiles of the causative pathogenic *E. coli* must first be analyzed (Fairbrother *et al.*, 2005). In this study, we surveyed 196 pathogenic *E. coli* isolates during six years after the ban on AGPs for their virulence and antimicrobial resistance profiles.



Fig. 1: Map of *Escherichia coli* isolation sites. Ninety-eight different pig herds (50 to 100 sows per herd) were located in three areas: northern (blue-colored; 21 farms in the Gangwon, Gyeonggi, and Incheon provinces), central (green-colored; 47 farms in the Chungbuk, Chungnam, and Gyeongbuk provinces), and southern (red-colored; 30 farms in the Chonbuk, Chonnam, and Gyeongnam provinces) South Korea.

There are various types of virulence factors in *E. coli*, and these virotypes are prone to geographical and temporal variations (Fairbrother *et al.*, 2012). In late 1990s, the most predominant fimbriae in Korea was F6, which then changed to F5 in the mid-2000s (Kwon *et al.*, 1999; Kwon *et al.*, 2002; Lee *et al.*, 2009). However, the most prevalent fimbria detected in this study was F18 (39.8%). The use of autovaccines or commercial vaccines could arise the shifts in the prevalence fimbriae (Byun JW *et al.*, 2013). In Korea, F4 and F18 targeting inactivated vaccines are being used nationwide (Chae *et al.*, 2012). Use of these vaccines could arise the antigenic variations, and it would account for the prevalence of fimbriae or non-fimbrial adhesins besides F4 and F18 in pigs.

In this study, we found that AIDA-1 (39 of 196 isolates, 33.1%) is usually detected in isolates from Korea and that AIDA-1 encoding *E. coli* strains are highly associated with F18 (odds ratio: 1.99, data not shown). Association between AIDA-1 and F18 was also reported by Niewerth *et al.*, (2001), Zhang *et al.*, (2007), and Zhao *et al.*, (2009). It has been hypothesized that AIDA-1 could affect the pigs and humans simultaneously, and this could be a problem from the perspective of public health (Zhao *et al.*, 2009). Considering that grower and finisher pigs are closely associated with slaughtering houses and meats, careful monitoring of AIDA-1 is imperative.

Unlike AIDA-1, *paa* gene is known to be highly associated with F4 (Zhang *et al.*, 2007, Byun JW *et al.*, 2013). In this study, we found that 17.0% of *paa*-encoding *E. coli* also encoded the F4 gene (data not shown). *Paa* protein, which was originally identified in a porcine enteropathogenic *E. coli* (EPEC), was known to play a role in attaching and effacing lesion (Fairbrother *et al.*,

2012). The *Paa* gene was detected at a high frequency in samples collected from 2011 to 2013. However, only two of the isolates collected between 2014 and 2016 were found to encode *paa* adhesins. This result indicates a decrease in the incidence rate of EPEC and attaching and effacing lesions in grower-finisher pigs in Korea. As mentioned above, F4 and F18 targeting inactivated vaccines are used nationwide (Do *et al.*, 2020a), and this could result in a decrease in *paa*, which is associated with F4. Although the specific role of the *paa* gene in the development of pathogenic *E. coli* has not yet been clearly defined, we hypothesize that horizontal gene transfer of the *paa* may arise the variability of virotypes (Leclerc *et al.*, 2007).

In the swine intestinal mucous membrane, *eae* gene develop the intimate adherence and it could arise the attaching and effacing lesions. It has also been previously detected in EPEC (Alonso *et al.*, 2017). In this study, no *eae*-positive isolates were identified.

In 1999 and 2000, the most prevalent enterotoxin gene was STa gene in Korea (Do *et al.*, 2019a). However, in this study, we found that Stx2e (44.9%) was the most frequently detected toxin gene, followed by STb (43.9%).

Stx2e gene leads the production of Shiga toxin variants 2e, and results in the onset of edema disease (Fairbrother *et al.*, 2012). These results indicate that there is a high incidence of edema disease in grower-finisher pigs in Korea. Interestingly, we found the time-dependent changes in the prevalence of the enterotoxin genes. The frequencies of most toxin genes, with the exception of STa, decreased in the period 2014–2016 compared to those in the period 2011–2013. Depending on the virulence factors encoded by infective *E. coli*, the clinical symptoms could vary. For example, the heat-stable toxin STa, which is produced by ETEC, causes osmotic diarrhea in pigs (Fairbrother *et al.*, 2005).

E. coli shows various virotypes, which is the combinations of virulence factors. In this study, the predominant virotypes remained unchanged. Virotypes STa:STb contained no colonization factors. It can be postulated that the use of vaccines targeting the frequently detected adhesins may lead the arise of new colonization factors not examined in this study (Do *et al.*, 2019a).

The isolates in this study showed extremely high resistance to ampicillin (93.9%), cephalothin (96.4%), tetracycline (87.2%), chloramphenicol (85.7%), and streptomycin (81.1%), similar to the monitoring results of Denmark (DANMAP, 2013), Canada (Government of Canada, 2014), and Japan (JVARM, 2016). In Korea, those antimicrobials have been extensively used by large quantities in the pig industry (Animal and Plant Quarantine Agency, 2019). There are other reports shows the similar results. High resistance against tetracycline (97.8%), ampicillin (89.1%) was reported by Cho *et al.*, (2006). And also, Lim *et al.*, (2014) reported *E. coli* isolates showed the high resistance to tetracycline (76.1%), ampicillin (64.6%), and streptomycin (58.4%). However, in this study, the reported resistance rates were higher than those reported by Lim *et al.*, (2014). This higher resistance rates may be resulted from differences in the origin of isolates. According to the Korean National Antimicrobial Resistance Monitoring System, bacteria

from diseased animals tend to show more antimicrobial resistance than from the normal livestock (Animal and Plant Quarantine Agency, 2019). Lim *et al.*, (2014) analyzed the resistance rates of *E. coli* isolated from normal livestock, whereas in this study, we analyzed the pathogenic *E. coli* from pigs showing diarrhetic symptoms.

During the six years after the ban on AGPs (2011–2016), resistance rates to gentamicin significantly decreased (from 52.3% to 10.1%). However, an increase in resistance rates against colistin (32.7% to 62.9%) and cefepime (7.5% to 16.9%) was observed. Antimicrobial resistance depends on the level of antimicrobial usage (Lim *et al.*, 2014). The sales for aminoglycosides (gentamicin) decreased from 58,975 kg (2010) to 48,218 kg (2016). Otherwise, the sales for cephalosporins (cefepime) increased from 4,980 kg (2010) to 9,623 kg (2016) (Animal and Plant Quarantine Agency, 2019). This symptom could account for the changes of resistance rates of the isolates.

Colistin has been regularly used to treat enteric colibacillosis because the colistin-resistant bacteria occur rarely and colistin resistance gene is hard to transfer horizontally. Colistin has been classified as one of the “highest priority critically important antimicrobials” in human by the World Health Organization (WHO) (Do *et al.*, 2019b). However, the *mcr* gene which is the plasmid-mediated colistin resistance gene was reported in Korea recently (Do *et al.*, 2020b). This *mcr* gene could arise the increase of colistin resistance. Increased colistin resistance could arise serious problems in veterinary medicine, and also in public health science. Thus, nationwide restrictions on the use of colistin are needed to reduce the colistin resistance.

In this study, majority of isolates showed multidrug resistance (192 out of 196 isolates, 98.0%). In comparison to the result (30.9%) of US pig origin with *E. coli* infections, the Korean pig origin isolates showed very higher multidrug resistance rates (90.7%) (Magiorakos *et al.*, 2011). Due to the lack of strict regulations on the use of antimicrobials in Korea, as opposed to other developed countries, using antimicrobials indiscriminately by non-specialists like livestock workers could increase the multidrug resistance rates (Do *et al.*, 2019b).

Between 2011 and 2013, no isolates resistant to 12 antimicrobial subclasses were detected. However, four such isolates were found between 2014 and 2016. This is thought to have resulted from the indiscriminate use of antimicrobials. In Korea, antibiotics for the growth promoting was entirely banned in July 2011 to manage antimicrobial resistance. And in August 2013, veterinarian prescriptions were introduced.

In this study, we compared the colonization factors, toxin genes, and antimicrobial resistance of *E. coli* from Korean diarrhetic grower and finisher pigs after the ban on AGPs. We confirmed that the most prevalent toxin gene shifted from Stx2e (56.1%) in 2011–2013 to STb (41.6%) in 2014–2016, and that there was a significant decrease in the prevalence of the colonization factor *paa*. Moreover, the trends in our findings suggest an increase in resistance to most antimicrobial agents followed after the ban on AGPs. These results provide important data for analyzing the impact of banning AGPs on the colonization factors and antimicrobial resistance of *E. coli*. It can be used for

the development of prevention and treatment strategies against enteric colibacillosis.

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

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