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

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**A B S T R A C T**

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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http://dx.doi.org/10.29261/pakvetj/2019.021

**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), enteraggregative *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 (bioMérieux, 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 antiserum purchased from SSI (Serum State 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), cefepine (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).

**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-antisera, 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

<table>
<thead>
<tr>
<th>O-serogroup</th>
<th>F4</th>
<th>F18</th>
</tr>
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<tbody>
<tr>
<td>O149*</td>
<td>33 (36.3%)</td>
<td>31 (93.9%)</td>
</tr>
<tr>
<td>O139*</td>
<td>2 (2.2%)</td>
<td>1 (50.0%)</td>
</tr>
<tr>
<td>O157</td>
<td>7 (7.7%)</td>
<td>7 (100.0%)</td>
</tr>
<tr>
<td>Others</td>
<td>4 (4.51%)</td>
<td>34 (82.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>94 (100.0%)</td>
<td>80 (87.2%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hemolysis</th>
<th>No. (%)</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%)</td>
<td>Hemolysis</td>
<td></td>
</tr>
<tr>
<td>O149*</td>
<td>4 (2.2%)</td>
<td>3 (75.0)</td>
</tr>
<tr>
<td>O139*</td>
<td>30 (16.6)</td>
<td>25 (83.3)</td>
</tr>
<tr>
<td>O157</td>
<td>5 (2.8)</td>
<td>5 (100.0)</td>
</tr>
<tr>
<td>Others</td>
<td>77 (42.5)</td>
<td>73 (94.8)</td>
</tr>
<tr>
<td>Total</td>
<td>180 (100.0)</td>
<td>181 (100.0)</td>
</tr>
</tbody>
</table>

**Significant difference between F4* and F18* *E. coli* (P<0.01). a No. of O-serogroup isolates / No. of F4* or F18* Escherichia coli isolates × 100 (%) b No. of hemolytic O-serogroup isolates / No. of O-serogroup isolates × 100 (%) c 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 d O-rough: non-typeable e O-typeable.
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 F4+ E. coli (26.4% and 8.8%, respectively).

Compared to the F4+ E. coli, the multidrug resistance rate of F18+ E. coli was significantly higher. Among the 12 subclasses of antibiotics used, the multidrug resistance rates of F4+ and F18+ E. coli were compared, and 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%), cefotaxim (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 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 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 fimbra. 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.

Acknowledgements: This study was supported by “Agriculture and life science industry development business” of Institute of Planning and Evaluation for Technology in Food, Agriculture, and Forestry in Ministry of Agriculture, Food, and Rural affairs. (Project No.: 114058-03-3-CG000).
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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