MICROBIOLOGY AND FOOD SAFETY

Molecular characterization of avian pathogenic *Escherichia coli* from broiler chickens with colibacillosis

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ABSTRACT Avian pathogenic *Escherichia coli* (**APEC**) causes extensive mortality in poultry flocks, leading to extensive economic losses. The aim of this study was to investigate the phenotypic and genotypic characteristics and antimicrobial resistance of recent APEC isolates. Of the 79 APEC isolates, the most predominant serogroup was O78 (16 isolates, 20.3%), followed by O2 (7 isolates, 8.9%) and O53 (7 isolates, 8.9%). Thirty-seven (46.8%) and six (7.6%) of the isolates belonged to phylogenetic groups D and B2, respectively, and presented as virulent extraintestinal *E. coli*. Among 5 analyzed virulence genes, the highest frequency was observed in *hlyF* (74 isolates, 93.7%), followed by *iutA* (72 isolates, 91.9%) gene. The distribution of the *iss* gene was significantly different between groups A/B1 and B2/D ($P < 0.05$). All group B2 isolates carried all 5 virulence genes. APEC isolates showed high resistance to ampicillin (83.5%), nalidixic acid (65.8%), tetracycline (64.6%), cephalothin (46.8%), and ciprofloxacin (46.8%). The β-lactamases–encoding genes $bla_{\text{TEM-1}}$ (23 isolates, 29.1%), $bla_{\text{CTX-M-1}}$ (4 isolates, 5.1%), and $bla_{\text{CTX-M-15}}$ (3 isolates, 3.8%); the aminoglycoside-modifying enzyme gene *aac(3)-II* (4 isolates, 5.1%); and the plasmid-mediated quinolone genes *qnrA* (10 isolates, 12.7%) and *qnrS* (2 isolates, 2.5%) were identified in APEC isolates. The *tetA* (37 isolates, 46.8%) and *sul2* (20 isolates, 25.3%) were the most prevalent among tetracycline and sulfonamide resistant isolates, respectively. This study indicates that APEC isolates harbor a variety of virulence and resistance genes; such genes are often associated with plasmids that facilitate their transmission between bacteria and should be continuously monitored to track APEC transmission in poultry farms.

Key words: avian pathogenic *Escherichia coli*, antimicrobial resistance, phylogenetic group, broilers

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INTRODUCTION

Colibacillosis is an infectious disease caused by avian pathogenic *Escherichia coli* (**APEC**) and affects poultry flocks worldwide including Korea (Kim et al., 2007; Lutful Kabir, 2010). APEC is associated with different kinds of disease ranging from respiratory tract infection to swollen head syndrome in poultry (Dho-moulin and Fairbrother, 1999). Avian colibacillosis primarily affects broiler chickens between the ages of 4 and 6 wk and is considered a principal cause of morbidity and mortality, leading to considerable economic losses to the poultry industry (Dho-moulin and Fairbrother, 1999; Guabiraba and Schouler, 2015).

APEC strains mostly belong to the phylogenetic group associated with extraintestinal pathogenic *E. coli* (**ExPEC**), but studies report wide serological diversity among strains (Wang et al., 2010a; Schouler et al., 2012). Although serogroups O2 and O78 represent 80% of disease-causing APEC worldwide (Dziva and Stevens, 2008), their prevalence varies among farms and countries described (Oh et al., 2011; Barbieri et al., 2015; Younis et al., 2017). A recent report characterized a set of APEC strains as *E. coli* isolates containing 2 or more virulence markers (Johnson et al., 2008). In particular, de Oliveira et al. (2015) reported that several virulence genes (*hlyF*, *ompT*, *iroN*, *iss*, and *iutA*) located on the large virulence-plasmid ColV were associated with APEC strains. In addition, Johnson et al. (2008) reported that APEC strains with virulence genes may act as zoonotic pathogens and virulence reservoirs and could jump to other species and cause human infection.

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In Korea, colibacillosis in broilers is a critically important disease and often occurs during respiratory stress caused by infection with *Mycoplasma gallisepticum* or viral agents such as infectious bronchitis virus (Oh et al., 2011). The use of antimicrobial drugs such as β-lactams, aminoglycosides, and fluoroquinolones remains the predominant option for colibacillosis outbreak (Kim et al., 2007). The continuous use of these antimicrobial drugs in poultry, however, has contributed to the emergence and sustenance of antimicrobial-resistant *E. coli* in Korea (Unno et al., 2011). Resistance in poultry bacteria may present a public health threat because of the potential transmission of resistance genes to human bacteria (Cavicchio et al., 2012).

Studies from several countries have documented the presence of virulence and resistance genes in APEC strains (Ahmeda et al., 2013; Ozaki et al., 2017). However, there have been only few systematic studies in Korea. This study investigated the phenotypic and genotypic characteristics and antimicrobial resistance in recent APEC isolates from commercial broiler farms in Korea.

MATERIALS AND METHODS

Bacterial Strains

In 2018, liver swab samples were obtained using transport media (Yuhan Lab tech, Seoul, Korea) from chickens presenting with colibacillosis lesions from 60 commercial broiler farms across the country. Isolation of *E. coli* was carried out according to the Processing and Ingredients Specification of Livestock Products published by the Ministry of Food and Drug Safety (2014). Swab samples were transported to the laboratory in a cooler and inoculated into mEC (Merck, Darmstadt, Germany). Subsequently, enriched samples were streaked onto MacConkey agar (BD Biosciences, Sparks, MD) and incubated at 37°C for 18 to 20 h. Suspected *E. coli* colonies were identified using PCR as previously described (Candrian et al., 1991). All *E. coli* isolates were analyzed using PCR as described by Johnson et al., (2008) as the minimal predictors of APEC virulence; *hlyF*, *iroN*, *iss*, *iutA*, and *ompT* genes (Table 1). If several isolates from the same farm demonstrated the same antimicrobial susceptibility patterns, one of these isolates was randomly selected for further study. A total of 79 APEC isolates were included in this study.

Serogrouping

O serogrouping was carried out using 162 primer pairs, from O1 to O187 as previously described (Iguchi et al., 2015).

Antimicrobial Susceptibility Testing

An antimicrobial susceptibility test was performed using the disc diffusion method, according to the standards and interpretive criteria described by the Clinical and Laboratory Standards Institute (CLSI, 2017). The following antimicrobials were used (BD Biosciences): amoxicillin-clavulanate (20/10 μg), ampicillin (10 μg), cefadroxil (30 μg), cefazolin (30 μg), cefepime (30 μg), cefotaxime (30 μg), cefoxitin (30 μg), ceftazidime (30 μg), cefuroxime (30 μg), cephalexin (30 μg), cephalothin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), imipenem (10 μg), nalidixic acid (30 μg), tetracycline (30 μg), and trimethoprim-sulfamethoxazole (1.25/23.75 μg). The *E. coli* strain ATCC 25922 was used for quality control purposes.

Detection of Antimicrobial Resistance Genes

Detection of antimicrobial resistance was performed with PCR using the primers listed in Table 1. Target antimicrobial resistance determinants were genes conferring resistance to β-lactam (*bla*TEM, *bla*SHV, *bla*OXA, and *bla*CTX), aminoglycoside [*aac(6′)-Ib, aac(3)-II*, and *ant(2″)-I*], plasmid-mediated quinolone (*qnrA*, *qnrB*, *qnrD*, *qnrS*, and *qepA*), tetracycline (*tetA*, *tetB*, and *tetC*), sulfonamide (*sul1* and *sul2*), and chloramphenicol (*catA1* and *cmlA*). β-Lactamase gene amplicons were sequenced with an automatic sequencer (Cosmogenetech, Seoul, Korea) and compared with those in the GenBank nucleotide database using the Basic Local Alignment Search Tool program available through the National Center for Biotechnology Information website (www.nici.nlm.nih.gov/BLAST).

Phylogenetic Groups

APEC phylogenetic grouping was accomplished by multiplex PCR-based phylogenetic typing method as described by Clermont et al. (2000). Groups were assigned as follows: Group A, *chuA*-negative and *TspE4.C2*-negative; Group B1, *chuA*-negative and *TspE4.C2*-positive; Group B2, *chuA* -positive and *yjaA*-positive; and Group D, *chuA*-positive and *yjaA*-negative.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Science version 25 (IBM, Seoul, Korea). The unpaired t test was used to investigate the relationship between phylogenetic groups and virulence genes from APEC isolates. A P value of less than 0.05 was considered statistically significant.

RESULTS

Serogrouping of APEC Isolates

Distribution of the O serogroups is shown in Table 2. Among 79 APEC isolates, 70 (88.6%) isolates were classified into 30 O serogroups, with 9 isolates remaining ungrouped. The most predominant serogroup was O78 (16 isolates, 20.3%), followed by O2 (7 isolates, 8.9%), O53

Table 1. Primers used for the amplification and DNA sequencing.

Primer	Sequence (5′ → 3′) Forward	Reverse	Base pair	Reference
Virulence genes				
iroN	AATCCGGCAAAGAGACGAACCGCCT	GTTCGGGCAACCCCTGCTTTGACTTT	553	Johnson et al., 2008
ompT	TCATCCCGGAAGCCTCCCTCACTACTAT	TAGCGTTTGCTGCACTGGCTTCTGATAC	496	Johnson et al., 2008
hlyF	GGCCACAGTCGTTTAGGGTGCTTACC	GGCGGTTTAGGCATTCCGATACTCAG	450	Johnson et al., 2008
Iss	CAGCAACCCGAACCACTTGATG	AGCATTGCCAGAGCGGCAGAA	323	Johnson et al., 2008
iutA	GGCTGGACATCATGGGAACTGG	CGTCGGGAACGGGTAGAATCG	302	Johnson et al., 2008
β-lactamases				
TEM	CATTTCCGTGTCGCCCTTATTC	CGTTCATCCATAGTTGCCTGAC	800	Dallenne et al., 2010
SHV	CACTCAAGGATGTATTGTG	TTAGCGTTGCCAGTGCTCG	885	Briñas et al., 2002
OXA	TTCAAGCCAAAGGCACGATAG	TCCGAGTTGACTGCCGGGTTG	702	Briñas et al., 2002
CTX-M group I	GACGATGTCACTGGCTGAGC	AGCCGCCGACGCTAATACA	499	Pitout et al., 2004
CTX-M group II	GCGACCTGGTTAACTACAATCC	CGGTAGTATTGCCCTTAAGCC	351	Pitout et al., 2004
CTX-M group III	CGCTTTGCCATGTGCAGCACC	GCTCAGTACGATCGAGCC	307	Pitout et al., 2004
CTX-M group IV	GCTGGAGAAAAGCAGCGGAG	GTAAGCTGACGCAACGTCTG	474	Pitout et al., 2004
Aminoglycoside-modifying enzymes				
aac(6′)-Ib	TGACCTTGCGATGCTCTATG	TTAGGCATCACTGCGTGTTC	508	Jiang et al., 2008
aac(3)-II	TGAAACGCTGACGGAGCCTC	GTCGAACAGGTAGCACTGAG	369	Sandvang and Aarestrup, 2000
ant(2″)-I	GGGCGCGTCATGGAGGAGTT	TATCGCGACCTGAAAGCGGC	740	Sandvang and Aarestrup, 2000
Plasmid-mediated quinolone				
qnrA	TCAGCAAGAGGATTTCTCA	GGCAGCACTATTACTCCCA	627	Wang et al., 2003
qnrB	CGACCTGAGCGGCACTGAAT	TGAGCAACGATGCCTGGTAG	515	Jiang et al., 2008
qnrD	CGAGATCAATTTACGGGGAATA	AACAAGCTGAAGCGCCTG	582	Cavaco et al., 2009
qnrS	ACCTTCACCGCTTGCACATT	CCAGTGCTTCGAGAATCAGT	571	Jiang et al., 2008
qepA	CGTGTTGCTGGAGTTCTTC	CTGCAGGTACTGCGTCATG	403	Minarini et al., 2008
Tetracyclines				
tetA	GTAATTCTGAGCACTGTCGC	CTGCCTGGACAACATTGCTT	956	Sengeløv et al., 2003
tetB	CTCAGTATTCCAAGCCTTTG	ACTCCCCTGAGCTTGAGGGG	414	Sengeløv et al., 2003
tetC	CCTCTTGCGGGATATCGTCC	GGTTGAAGGCTCTCAAGGGC	505	Sengeløv et al., 2003
Sulfonamide				
sul1	CTTCGATGAGAGCCGGCGGC	GCAAGGCGGAAACCCGCGCC	433	Sandvang et al., 1998
sul2	CGGCATCGTCAACATAACC	GTGTGCGGATGAAGTCAG	720	Maynard et al., 2003
Chloramphenicol				
catA1	AGTTGCTCAATGTACCTATAACC	TTGTAATTCATTAAGCATTCTGCC	547	Van et al. 2008
cmlA	CCGCCACGGTGTTGTTGTTATC	CACCTTGCCTGCCCATCATTAG	698	Van et al. 2008
Phylogenetic group				
chuA	GACGAACCAACGGTCAGGAT	TGCCGCCAGTACCAAAGACA	279	Clermont et al., 2000
yjaA	TGAAGTGTCAGGAGACGCTG	ATGGAGAATGCGTTCCTCAAC	211	Clermont et al., 2000
TspE4C2	GAGTAATGTCGGGGCATTCA	CGCGCCAACAAAGTATTACG	152	Clermont et al., 2000

(7 isolates, 8.9%), O3 (3 isolates, 3.8%), O86 (3 isolates, 3.8%), and O174 (3 isolates, 3.8%).

Antimicrobial Resistance Profiles of APEC Isolates

The 79 APEC isolates showed high resistance to ampicillin (66 isolates, 83.5%), nalidixic acid (52 isolates, 65.8%), tetracycline (51 isolates, 64.6%), cephalothin, and ciprofloxacin (37 isolates, 46.8% for each). Resistance to the third generation cephalosporins cefotaxime and ceftazidime and the fourth generation cefepime was detected in 18 isolates (22.8%), 14 isolates (17.7%), and 5 isolates (6.3%), respectively (Figure 1).

Prevalence of Antimicrobial Resistance Genes

The prevalence of antimicrobial resistance genes is shown in Table 2. Twenty-eight (35.4%) APEC isolates carried the following β-lactamase encoding genes: $bla_{\text{TEM-1}}$ (23 isolates, 29.1%), $bla_{\text{CTX-M-1}}$ (4 isolates, 5.1%), and $bla_{\text{CTX-M-15}}$ (3 isolates, 3.8%). Three types of aminoglycoside-modifying enzyme genes were examined, but *aac(3)-II* was only found in 4 (5.1%) APEC isolates. Plasmid-mediated quinolone resistance genes were detected in 12 (15.2%) APEC isolates as follows: *qnrA* (10 isolates, 12.7%) and *qnrS* (2 isolates, 2.5%). The *qnrB*, *qnrD*, and *qeqA* genes were not detected. Among tetracycline resistance genes, *tetA* (37 isolates, 46.8%) was the most prevalent one, followed by *tetB* (10 isolates, 12.7%) and *tetC* (2 isolates, 2.5%). The *sul1* and *sul2* sulfonamide resistance genes were detected in 5 isolates (6.3%) and 20 isolates (25.3%), respectively. The *catA1* and *cmlA* chloramphenicol resistance genes were each found in 5 isolates (6.3%).

Phylogenetic Groups and Virulence Genes of APEC Isolates

Distributions of phylogenetic groups and virulence genes are shown in Table 3. Thirty-seven (46.8%) and six (7.6%) of the isolates belonged to groups D and B2, respectively, and presented as virulent extraintestinal *E. coli*. The 16 isolates from the predominant O78 serogroup were divided into groups A (8 isolates,

Table 2. Distribution of serotypes and antimicrobial resistance genes of avian pathogenic *E. coli*.

O serogroup	No. of isolates	No. of isolates carried target gene (%)												
		β-Lactamases			Aminoglycoside-modifying enzymes	Plasmid-mediated quinolone		Tetracyclines			Sulfonamide		Chloramphenicol	
		bla$_{\text{TEM-1}}$	*bla*$_{\text{CTX-M-1}}$	*bla*$_{\text{CTX-M-15}}$	*aac (3)-II*	*qnrA*	*qnrS*	*tetA*	*tetB*	*tetC*	*sul1*	*sul2*	*catA1*	*cmlA*
O78	16	6 (37.5)	-	-	3 (18.8)	1 (6.3)	-	6 (37.5)	3 (18.8)	-	1 (6.3)	3 (18.8)	1 (6.3)	-
O2	7	-[5]	-	-	-	-	-	4 (57.1)	-	-	-	3 (42.8)	-	-
O53	7	-	-	-	1 (14.3)	-	-	-	-	-	-	-	-	-
O3	3	1 (33.3)	-	-	-	-	-	-	-	-	-	1 (33.3)	-	-
O86	3	-	-	-	-	1 (33.3)	-	3 (100.0)	1 (33.3)	-	-	-	-	1 (33.3)
O174	3	-	3 (100.0)	-	-	-	-	3 (100.0)	-	-	-	-	-	-
O8	2	-	-	1 (50.0)	-	-	-	1 (50.0)	-	-	-	-	-	-
O9	2	2 (100.0)	-	1 (50.0)	-	2 (100.0)	-	2 (100.0)	-	-	1 (50.0)	-	-	-
O25	2	2 (100.0)	-	-	-	-	-	2 (100.0)	-	-	1 (50.0)	1 (50.0)	-	-
O45	2	1 (50.0)	-	-	-	-	-	-	1 (50.0)	1 (50.0)	-	1 (50.0)	1 (50.0)	-
O115	2	-	-	-	-	1 (50.0)	-	1 (50.0)	-	-	-	1 (50.0)	2 (100.0)	-
O132	2	-	-	-	-	1 (50.0)	-	1 (50.0)	-	-	1 (50.0)	-	-	-
O142	2	1 (50.0)	-	-	-	-	-	-	1 (50.0)	-	-	2 (100.0)	-	1 (50.0)
O11	1	1 (100.0)	-	-	-	-	-	-	1 (100.0)	-	-	1 (100.0)	-	-
O37	1	-	-	-	-	-	-	-	-	-	-	-	-	-
O60	1	-	-	-	-	-	1 (100.0)	1 (100.0)	-	-	-	1 (100.0)	-	-
O76	1	1 (100.0)	-	-	-	-	-	-	-	-	-	-	-	-
O88	1	1 (100.0)	1 (100.0)	-	-	1 (100.0)	-	1 (100.0)	-	-	-	1 (100.0)	-	-
O99	1	-	-	-	-	-	-	1 (100.0)	-	-	1 (100.0)	1 (100.0)	-	-
O103	1	1 (100.0)	-	-	-	-	1 (100.0)	-	1 (100.0)	-	-	-	-	-
O104	1	-	-	-	-	-	-	-	-	-	-	-	-	-
O111	1	-	-	-	-	-	-	-	-	-	-	-	-	-
O146	1	-	-	-	-	-	-	-	1 (100.0)	-	-	-	-	-
O158	1	1 (100.0)	-	-	-	-	-	1 (100.0)	-	-	-	1 (100.0)	-	-
O161	1	-	-	-	-	-	-	1 (100.0)	-	-	-	-	-	-
O166	1	-	-	-	-	-	-	1 (100.0)	-	-	-	-	1 (100.0)	-
O173	1	1 (100.0)	-	-	-	-	-	1 (100.0)	-	-	-	1 (100.0)	-	-
Ogp6[1]	1	1 (100.0)	-	-	-	-	-	1 (100.0)	-	-	-	-	-	-
Ogp8[2]	1	-	-	-	-	1 (100.0)	-	1 (100.0)	-	-	-	1 (100.0)	-	-
Ogp14[3]	1	1 (100.0)	-	-	-	-	-	1 (100.0)	-	-	-	-	-	-
ONT[4]	9	2 (22.2)	-	1 (11.1)	-	2 (22.2)	-	4 (44.4)	1 (11.1)	1 (11.1)	-	1 (11.1)	-	3 (33.3)
Total	79	23 (29.1)	4 (5.1)	3 (3.8)	4 (5.1)	10 (12.7)	2 (2.5)	37 (46.8)	10 (12.7)	2 (2.5)	5 (6.3)	20 (25.3)	5 (6.3)	5 (6.3)

[1]O46 or O134.
[2]O107 or O117.
[3]O62 or O68.
[4]Not grouped.
[5]Not detected.

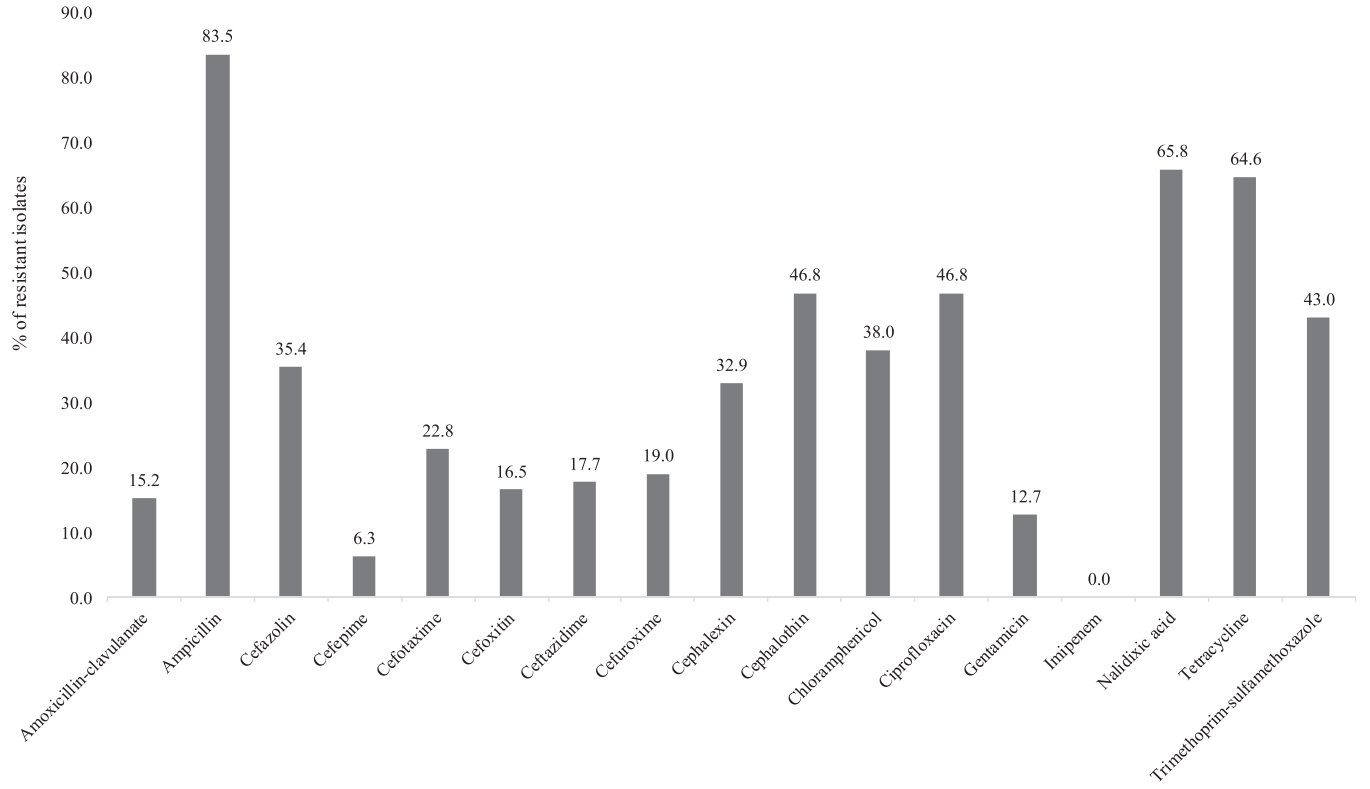

Figure 1. Prevalence of antimicrobial resistance of avian pathogenic *E. coli*.

50.0%), B1 (4 isolates, 25.0%), and D (4 isolates, 25.0%). O2 and O53, followed by predominant serogroups, belonged to groups B2 or D. The virulence gene found most often was *hlyF* (74 isolates, 93.7%), followed by *iutA* (72 isolates, 91.9%), *ompT* (71 isolates, 89.9%), *iroN* (63 isolates, 79.9%), and *iss* (62 isolates, 78.5%). The distribution of the *iss* gene varied significantly between groups A/B1 and B2/D ($P < 0.05$). All group B2 isolates carried all 5 virulence genes.

DISCUSSION

APEC is associated with extraintestinal infections causing a range of disease known as colibacillosis in poultry. Although different O serogroups have been associated with colibacillosis, a limited number of serogroups, O1, O2, and O78, have been reported in chickens. In this study, O78 was the most predominant serogroup, followed by O2 which is similar to the results from previous studies (Wang et al., 2010b; Younis et al., 2017). ExPEC has a complex phylogenetic structure and carry a wide range of virulence factors (Sarowska et al., 2019). In particular, virulent extraintestinal stains are predominantly found in phylogenetic groups B2 and D, whereas commensal intestinal strains are found in groups A or B1 (Koga et al., 2015). Although only 25% of the O78 isolates belonged to group D, all O2 *E. coli* isolated in this study belonged to groups B2 or D. Serogroup O2 contain the most frequently isolated *E. coli* types from humans, along with serogroup O1 strains which were not detected in this study (Ciesielczuk et al., 2016). Delannoy et al. (2017) also reported that serogroup O2 *E. coli* from avian colibacillosis shares certain ExPEC virulence traits with human *E. coli* isolates. In this study, O53 was also a major serogroup, although it was not the predominant serogroup in the previous studies (Wang et al., 2010b; Solà-Ginés et al., 2015), and three isolates were grouped in O174, which was described in *E. coli* isolates from beef cattle (Mekata et al., 2014). In particular, all O53 and O174 isolates also belonged to phylogenetic groups B2 or D. These results indicate that the diversity of antigens presented by APEC may be members of a broad reservoir in domestic animals and humans.

Five essential virulence genes, *hlyF* (hemolysin), *ompT* (outer membrane protease), *iroN* (siderophore), *iss* (serum survival), and *iutA* (iron transport), are considered markers for APEC (de Oliveira et al., 2015; Jørgensen et al., 2019). Johnson et al. (2008) reported that APEC isolates from poultry clinically diagnosed with colibacillosis were positive for at least one of these 5 genes. In this study, all APEC isolates also carried at least one of these 5 genes, and the prevalence of genes was 78.5 to 93.7%. In particular, all the phylogenetic group B2 APEC isolates carried all 5 virulence genes, and the distribution of the *iss* gene was significantly different between groups A/B1 and B2/D ($P < 0.05$). The *iss* gene confers resistance to serum complement immune responses and increases the virulence of *E. coli* 100-fold in day-old chicks (Nolan et al., 2002). Presence of majority of APEC virulence genes, however, was independent of phylogenetic group.

Antimicrobial treatment has been considered to be an important determinant for reducing economic losses by

Table 3. Distribution of phylogenetic groups and virulence genes of avian pathogenic *E. coli*.

Phylogenetic group	No. of isolates (%)	No. of isolates with each virulence gene (%)					O Serotypes (no. of isolates)
		hlyF	*iroN*	*iss*[1]	*iutA*	*ompT*	
A	18 (22.8)	16 (88.9)	11 (61.1)	11 (61.1)	14 (77.8)	16 (88.9)	O3 (3), O8 (1), O9 (1), O78 (8), O99 (1), O103 (1), Ogp14 (1)[2], ONT (2)[3]
B1	18 (22.8)	18 (100.0)	15 (83.3)	12 (66.7)	17 (94.4)	16 (88.9)	O37 (1), O76 (1), O78 (4), O86 (1), O88 (1), O115 (2), O132 (1), O173 (1), Ogp6 (1)[4], ONT (5)
B2	6 (7.6)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	O2 (5), O104 (1)
D	37 (46.8)	34 (91.9)	31 (83.8)	33 (89.2)	35 (94.6)	33 (89.2)	O2 (2), O8 (1), O9 (1), O11 (1), O25 (2), O45 (2), O53 (7), O60 (1), O78 (4), O86 (2), O111 (1), O132 (1), O142 (2), O146 (1), O158 (1), O161 (1), O166 (1), O174 (3), Ogp8 (1)[5], ONT (2)
	79	74 (93.7)	63 (79.7)	62 (78.5)	72 (91.1)	71 (89.9)	

[1]There were significant differences ($P < 0.05$) between A and B1 and between B2 and D.
[2]O62 or O68.
[3]Not grouped.
[4]O46 or O134.
[5]O107 or O117.

colibacillosis. The APEC isolates from this study were resistant to antimicrobials critically important to human medicine, such as cephalosporins and fluoroquinolones, and also showed resistance to antimicrobials such as ampicillin and tetracycline. In particular, cephalosporin-resistant isolates from poultry industries have been on the rise (APQA, 2017), and many β-lactamase–encoding genes have been described (Dallenne et al., 2010; Seo et al., 2019). In this study, 2 groups of β-lactamase genes were identified, of which $bla_{\text{TEM-1}}$ was the most prevalent. The $bla_{\text{TEM-1}}$ is widespread in *E. coli* from the poultry industry, including APEC isolates in Korea, but only codes for narrow-spectrum β-lactamases that can inactivate penicillins and aminopenicillins (Kim et al., 2007; Poirel et al., 2008; Seo et al., 2019). Extended-spectrum β-lactamases such as CTX-M that hydrolyze the characteristic β-lactam ring are of greater concern because they confer resistance to most β-lactam antimicrobials, including cephalosporins (Paterson and Bonomo, 2005), and can lead to increased resistance to other antimicrobials via horizontal gene transfer (Zurfluh et al., 2014; Hoepers et al., 2018). The $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$ were also detected in this study. Although these genes have been previously reported in *E. coli* isolates from poultry industry (Jo and Woo, 2016; Seo et al., 2019), this study is the first to report detection of these genes in APEC in Korea.

Fluoroquinolones are also important antimicrobial agents for treating various types of infections in both humans and animals (Mellata, 2013; Dandachi et al., 2018). Several plasmid-encoded resistance genes related with Qnr-like proteins (*qnrA*, *qnrD*, and *qnrS*) which protect DNA from quinolone binding, the *aac(6′)-Ib-cr* acetyltransferase that modifies certain fluoroquinolones, and active efflux pumps (*qepA*) have been found in fluoroquinolone-resistant isolates (Poirel et al., 2008; Rodríguez-Martínez et al., 2016). In this study, only *qnrA* and *qnrS* were identified; however, these genes can be transferred to other strains by conjugative plasmids.

Aminoglycosides are frequently used in poultry industry in Korea (APQA, 2017), particularly gentamicin, which is commonly injected subcutaneously to vaccinate against Marek's or bursal disease in day-old chicks in hatcheries (APQA, 2017; Kim et al., 2018). The most common mechanism of aminoglycoside resistance is chemical modification by aminoglycoside-modifying enzyme genes (Garneau-Tsodikova and Labby, 2015). In this study, the *aac(3)-II* gene that encodes *N*-acetyltransferases was identified in 4 APEC isolates. Resistance to gentamicin is generally found in less than 20% of APEC isolates, similar to the prevalence of gentamicin-resistant *E. coli* isolated from poultry in Korea (APQA, 2017).

According to reports from the National Antimicrobial Resistance Monitoring Program, tetracyclines are the leading antimicrobials purchased in Korea (AQPA, 2017). In this study, 37 of the 51 tetracycline-resistant APEC isolates carried the *tetA* gene. The *tetA* and *tetB* genes encode efflux mechanisms and are the most common tetracycline resistance determinant in *E. coli* (Van et al., 2008; Diarra et al., 2016). The relative prevalence of *tetA* was higher than that of *tetB* or *tetC* in Korea (Kim et al., 2007; Dessie et al., 2013).

Sulfonamide resistance is conferred by *sul1* and *sul2* (Shin et al., 2014). In this study, the *sul2* gene was detected in a larger proportion of the isolates, and the more frequent presence of *sul2* than *sul1* has also been reported in previous studies about *E. coli* from poultry industry (Guerra et al., 2003; Drugdová and Kmeť, 2013). The *sul1* gene, however, is often found together with other antimicrobial resistance genes in gene cassettes in the carriable components of class 1 integrons (Poirel et al., 2008).

Chloramphenicol resistance is mediated enzymatically by the plasmid-located chloramphenicol acetyltransferase gene *catA1* and nonenzymatically by the chloramphenicol resistance gene *cmlA* (Kikuvi et al., 2007). In this study, 10 of the 30 chloramphenicol-resistant APEC isolates carried *catA1* or *cmlA*, and these genes

may also be cotransferred to bacteria with other antimicrobial resistance genes as previously reported (Travis et al., 2006). This study characterized a wide diversity of serogroups, antimicrobial resistance, and virulence properties in recent APEC isolates, and we recommend continuous monitoring to track APEC transmission in poultry farms.

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