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Research article

Extended-spectrum beta-lactamase (ESBL) production and virulence genes profile of avian pathogenic *Escherichia coli* (APEC) isolated from broiler chickens in eastern Thailand

Suttitas Tongkamsai[1,*] and Kulchai Nakbubpa[1]

[1]*Faculty of Veterinary Medicine, Rajamangala University of Technology Tawan-ok, Chonburi, 20110 Thailand.*

Abstract

Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis, resulting in extensive economic losses for the broiler industry. To date, there is little information in Thailand about virulence-associated genes and antibiotic resistance of APEC strains. Here, this study aimed to investigate the virulence genes and extended-spectrum beta-lactamase (ESBL) characteristics of APEC isolated from broilers. This study used multiplex polymerase chain reaction (PCR) to determine the presence of virulence genes and resistance genes in APEC. Furthermore, the disc diffusion method examined ESBL phenotypes of APEC and antibiotic resistance profiles against 17 antimicrobials. In this study, *E. coli* was isolated 11 (6.32%) of 174 broiler visceral organs. All *E. coli* isolates were tested for five APEC-virulence-associated genes (*iroN, ompT, hlyF, iutA,* and *iss*). Eight E. coli isolates from broilers with colibacillosis-associated lesions carried two (*iroN* and *ompT*) of the APEC virulence genes. One APEC virulence gene (*hlyF*) was found in *E. coli* isolates from broilers without lesions. Possibly no individual virulence gene was specific to the APEC strain. Interestingly, the *papC*, previously detected in humans with uropathogenic *E. coli*, was found in an APEC isolate. All APEC isolates were ESBL-producing *E. coli*, and then they were tested for four beta-lactamase-encoding genes (blaTEM, blaCTX-M, blaOXA, and blaSHV). The blaTEM and blaCTX-M were identified in 81.81% (9/11) of the isolates, whereas blaOXA or blaSHV were not detected in any isolate. All APEC showed multi-drug resistance (MDR) phenotypes, especially chloramphenicol, erythromycin, and sulfamethoxazole-trimethoprim. Although antibiotics were not recently used, MDR might be encouraged by horizontally transferring antibiotic-resistance genes. In addition, fluoroquinolone resistances were found in APEC isolates which could transfer resistance genes to humans via the food chain. This study indicates that APEC isolates contain several virulence and *bla* genes and should be surveillance to prevent the transmission of those genes to humans.

Keywords: Avian pathogenic Escherichia coli, Extended-spectrum beta-lactamase, Molecular characteristics

***Corresponding author:** Suttitas Tongkamsai, Faculty of Veterinary Medicine, Rajamangala University of Technology Tawan-ok, Chonburi 20110, Thailand. E-mail: suttitas_to@rmutto.ac.th.

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INTRODUCTION

Escherichia coli (*E. coli*) is a facultative anaerobic microorganism that is present in the normal microflora in human and poultry intestines (Klimienė et al., 2017; Joseph et al., 2023). In particular, avian pathogenic *E. coli* (APEC) can enrich specific virulence genes that spread into various internal organs to cause disease (Won et al., 2009). APEC caused colibacillosis, resulting in significant economic losses for the broiler industry worldwide—due to morbidity, costs acquired in disease control, and carcass condemnations at the slaughterhouse. The disease's clinical manifestation is mainly associated with airsacculitis and fibrinous polyserositis (perihepatitis, pericarditis, and peritonitis) (Azam et al., 2019). The pathogenicity of APEC has been explained by the occurrence of virulence factors, including the aero actin receptor, encoded by the gene *iutA*; the hemolysin F, encoded by *hlyF* gene; the increased serum survival, encoded by the gene *iss*; the enterobactin siderophore receptor protein, encoded by the gene *iroN* and the outer membrane protease, encoded by the *ompT*. A recent report showed that the most associated with APEC strains were *E. coli* isolates carrying those five virulence genes (Kim et al., 2020). Additionally, the temperature-sensitive hemagglutinin (*tsh*) gene promotes the air sac lesion by raising the colonization rate. This gene has been proven that associated with yolk sac infection and colisepticemia in chickens (Rezatofighi et al., 2021). The ability of APEC to prevent them from the immune response and survive the extra-intestinal tract of the host has been proven to be associated with the iron acquisition (*iucC*) gene. The P fimbriae (*papC*) found in both APEC and human pathogenic *E. coli* indicated that APEC might spread virulence to humans (Sola-Gines et al., 2015). Indeed, several virulence factors have been implicated with APEC strains, although the mechanisms underlying pathogenicity are not well known. In livestock production, antibiotics are widely used as a growth promoter and in preventing diseases. Currently, antibiotics such as beta-lactam, tetracycline, and sulfonamide remain successful in treating avian colibacillosis (Yoon and Lee, 2022). However, prolonged medication has contributed to the high resistance to antibiotic drugs of *E. coli*. In particular, beta-lactam is considered for colibacillosis outbreak, leading to the spread of APEC-producing extended-spectrum beta-lactamases (ESBL) worldwide (Hiroi et al., 2012). The most common ESBL variants in *E. coli* are TEM, CTX-M, OXA, and SHV, while the $bla_{\text{CTX-M}}$ family has been reported mainly from poultry. ESBL-producing APEC may present a public health problem because of the dissemination of drug-resistance genes to human bacteria, such as third-generation cephalosporin resistance (Geser et al., 2012). Several studies have described the presence of virulence and ESBL-resistance genes in APEC strains. However, a previous publication reported a negative correlation between the presence of virulence and antibiotic-resistance genes (Tohmaz et al., 2022). So far, few studies have been combined with colibacillosis-associated lesions and antimicrobials used. For this reason, this study aimed to investigate the characteristics of virulence genes and ESBL-producing APEC strains isolated from broiler farms in eastern Thailand.

MATERIALS AND METHODS

Sample collection and isolation of *E. coli* strains

E. coli was isolated from 174 broiler chickens from 3 chicken farms in eastern Thailand. CNP and KN farms are commercial broiler farms in Chonburi and Chachoengsao provinces, respectively. SY farm is an antibiotic-free broiler farm established three years ago in Rayong province. Fifty-eight chickens were randomly collected from each farm and examined for the gross lesion of colibacillosis by postmortem. Liver and heart samples were collected separately and a loopful of inoculum through the heat-sterilized surface was streaked on MacConkey agar (Aberkane et al., 2023). The plates were incubated under aerobic conditions at 37°C for 18-24 hours. Bacterial growth was observed and one lactose fermenter colony was subjected to biochemical identification (Rahman et al., 2020). The *E. coli* isolates' biochemical tests were done as oxidase-negative, indole-positive, and H_2S-negative. Subsequently, *E. coli* isolates were selected and stored in 20% glycerol stock at -80°C for further molecular studies (16s rRNA and ESBL-encoding genes detection). Ethical approval for collecting animal samples was approved by Institutional Animal Care and Use Committee (RMUTTO-ACUC-2-2023-007).

Antimicrobial susceptibility testing

Antimicrobial testing was carried out by the disc diffusion method against 17 antimicrobial agents (Oxoid, England) according to CLSI guidelines (CLSI, 2023). They included Ampicillin (AMP, 10µg), Amoxycillin (AML, 20µg), Amoxicillin/clavulanate (AMC, 20/10µg), Aztreonam (ATM, 30µg), Chloramphenicol (C, 30µg) Ciprofloxacin (CIP, 5µg), Doxycycline (DO, 30µg), Enrofloxacin (ENR, 5µg), Erythromycin (E, 15µg), Gentamicin (CN, 10µg), Imipenem (IPM, 10µg), Kanamycin (K, 30µg), Oxytetracycline (OT, 30µg), Polymyxin B (PB, 30U), Sulfamethoxazole-trimethoprim (SXT, 1.25/23.75µg), Streptomycin (S, 10µg), and Tetracycline (TET, 30µg). In brief, test bacteria were subcultured on nutrient agar (Himedia, India) for 24 hours at 37°C. Colonies were suspended in 5 ml sterile normal saline and the suspension density was adjusted to 0.5 McFarland turbidity standards. Sterile cotton swabs were dipped into the adjusted suspension and then swabbed on Muller-Hinton agar (MHA) (Difco, USA) plates (Eltai et al., 2020). Antimicrobial discs were plated on the MHA plates and incubated at 37°C for 24 hours. After incubation, the inhibition zone (mm.) diameter around each antimicrobial disc was measured. The results were interpreted as sensitive, intermediate, and resistant according to CLSI criteria (CLSI, 2023). The *E. coli* TISTR 527 (original code: ATCC 11775) was used for quality control. The bacteria strain was obtained from the Thailand Institute of Scientific and Technological Research and previously tested for antimicrobial activity (Rakkhumkaew and Pengsuk, 2018). Multidrug-resistant (MDR) isolate was defined as resistance to at least one agent in three or more different classes of antibiotics (Magiorakos et al., 2012).

ESBL screening, confirmation of ESBL production, and detection of ESBL genes

Presumptive ESBL production was performed for all isolates by standard disc diffusion method using cefotaxime (CTX, 30 ug) and ceftazidime (CAZ, 30ug). When isolates were resistant to third-generation cephalosporins, they were consequently confirmed for ESBL production. ESBL production was phenotypically identified using the double disk synergy test using one β-lactamase inhibitor (AMC) disc and two cephalosporin discs (CAZ and CTX). As previously described, three discs were placed at a 20 mm distance (center to center) on MHA plates (Anago et al., 2015). The positive-ESBL production was interpreted as isolates resistant to CAZ and/or CTX and with an increase in the size of the zone diameter toward the AMC. The template DNA was prepared using the boiling method (Li et al., 2012). Briefly, a few colonies were suspended in 1 mL of Nuclease-free water and boiled at 100°C for 10 min. The suspension was centrifuged at 10,000 x g for 2 min. and stored at -20°C before use. The presence of ESBL-encoding genes (bla_{TEM}, bla_{CTX-M}, bla_{SHV}, and bla_{OXA}) and 16s rRNA gene of *E. coli* were detected by multiplex PCR in a single tube with specific primers (Table 1). For amplification, 3 µL of DNA template was mixed with 10 pmol of each primer, 12.5 µL PCRBIO Taq Mix Red (PCRBIOSYSTEMS, UK), and Nuclease-free water in a final volume of 25 µL under optimized conditions as described previously (Kamaruzzaman et al., 2020). The PCR product was separated on a 1.5% agarose gel electrophoresis and was visualized with a gel documentation system. The positive control used in the current study was *E. coli* TISTR 527, while Nuclease-free water was used as a negative control.

Detection of virulence gene

The virulence-associated genes of all *E. coli* isolates were searched using two sets of multiplex PCR as follows: set 1 *iroN, ompT, hlyF, iutA,* and *papC*; set 2 *iss, tsh,* and *iucC*. The PCR reactions of each set were previously described (Skyberg et al., 2003; van der Westhuizen and Bragg, 2012), and the primers used in this study are summarized in Table 1. In addition, the amplicons were analyzed as described above.

Table 1 PCR primers used in this study

Gene	Sequence 5' to 3'	Size (bp)	References
16sRNA			
E. coli	F: TGACGTTACCCGCAGAAGAA R: CTCCAATCCGGACTACGACG	832	(Kamaruzzaman et al., 2020)
beta-lactamases			
bla_{TEM}	F: TCCTTGAGAGTTTTCGCCCC R: TGACTCCCCGTCGTGTAGAT	643	(Kamaruzzaman et al., 2020)
bla_{CTX-M}	F: AAGCACGTCAATGGGACGAT R: GTTGGTGGTGCCATAGCCA	402	(Kamaruzzaman et al., 2020)
bla_{SHV}	F: CAATCACGACGGCGGAATCT R: GTGGGTCATGTCGGTACCAT	168	(Kamaruzzaman et al., 2020)
bla_{OXA}	F: TTGCACTTGATAGTGGTGTGA R: AGTGAGTTGTCAAGCCAAAAAGT	250	(Kamaruzzaman et al., 2020)
Virulence genes			
iroN	F: AAGTCAAAGCAGGGGTTGCCCG R: GATCGCCGACATTAAGACGCAG	667	(van der Westhuizen and Bragg, 2012)
ompT	F: TCATCCCGGAAGCCTCCCTCACTACTAT R: TAGCGTTTGCTGCACTGGCTTCTGATAC	496	(van der Westhuizen and Bragg, 2012)
hlyF	F: GGCCACAGTCGTTTAGGGTGCTTACC R: GGCGGTTTAGGCATTCCGATACTCAG	450	(van der Westhuizen and Bragg, 2012)
iss	F: GTGGCGAAAACTAGTAAAACAGC R: CGCCTCGGGGTGGATAA	760	(Skyberg et al., 2003)
iutA	F: GGCTGGACATCATGGGAACTGG R: CGTCGGGAACGGGTAGAATCG	302	(van der Westhuizen and Bragg, 2012)
tsh	F: GGGAAATGACCTGAATGCTGG R: CCGCTCATCAGTCAGTACCAC	420	(Skyberg et al., 2003)
iucC	F: CGCCGTGGCTGGGGTAAG R: CAGCCGGTTCACCAAGTATCACTG	541	(Skyberg et al., 2003)
papC	F: TGATATCACGCAGTCAGTAGC R: CCGGCCATATTCACATAA	501	(Ewers et al., 2005)

RESULTS

APEC was isolated from 11 (6.32%) of 174 visceral organ samples. All APEC isolates were ESBL-producer *E. coli* (Table 2). Of these, 2 (18.18%) samples from an antibiotics-free farm and a tetracycline antibiotic-used farm were positive for ESBL-producing *E. coli*. The highest occurrence of ESBL-producing *E. coli* was 63.63% in a penicillin antibiotic-used farm. All APEC isolates were resistant to Chloramphenicol, Erythromycin, and Sulfamethoxazole-trimethoprim. High resistance rates were observed for Amoxycillin (90.90%), Oxytetracycline, Polymyxin B, and Tetracycline (81.81%), Ampicillin (72.72%), Doxycycline (45.45%) and Enrofloxacin (36.36%). Aztreonam gave resistance results specific to KNH4, and 318L resisted Kanamycin. The most common antimicrobial resistance patterns were AMP-C-E-TET-PB-SXT (3 isolates, 27.27%). A total of 9 isolates (81.81%) were found positive in combinations with bla_{TEM} and bla_{CTX-M} genes, whereas bla_{SHV} and bla_{OXA} genes were not detected in all 11 APEC isolates (Figure 1). Genotypic detection of ESBL genes was not found in isolates KN2 and SY301.

Table 2 Determinants of Antimicrobial resistance phenotype, genotype, and antimicrobial used among all 11 isolates

Isolate	Antimicrobial used	Antimicrobial resistance phenotype	ESBL-positive *E. coli* isolates	Beta-lactamases genes
CNP4	Doxycycline	AMP-C-E-TET-PB-SXT AML, OT	positive	TEM, CTX-M
CNP6		AMP-C-E-TET-PB-S-SXT AML, OT	positive	TEM, CTX-M
KN1	Amoxycillin	AMP-C-E-TET-PB-CN-SXT AML, OT, DO	positive	TEM, CTX-M
KN2		C-E-TET-ENR-PB-SXT OT, DO	positive	Not found
KN4		AMP-C-E-TET-PB-SXT AML, OT	positive	TEM, CTX-M
KNH1		AMP-C-E-TET-PB-SXT AML, OT, DO,	positive	TEM, CTX-M
KNH2		AMP-C-E-TET-SXT AML, OT, DO	positive	TEM, CTX-M
KN3		AMP-C-E-TET-CIP-PB- SXT, AML, ENR, OT	positive	TEM, CTX-M
KNH4		AMP-C-E-ENR-PB-SXT AML, ATM,	positive	TEM, CTX-M
SY301	Antibiotics-free	AML-C-E-PB-SXT	positive	Not found
SY318		AMP-C-E-TET-CIP-K-SXT AML, AMC, ENR, OT, DO	positive	TEM, CTX-M

AMP: Ampicillin; AML: Amoxycillin; AMC: Amoxicillin/clavulanate; ATM: Aztreonam; C: Chloramphenicol; CIP: Ciprofloxacin; DO: Doxycycline; ENR: Enrofloxacin; E: Erythromycin; CN: Gentamicin; IPM: Imipenem; K: Kanamycin; OT: Oxytetracycline; PB: Polymyxin B; SXT: Sulfamethoxazole-trimethoprim; S: Streptomycin; TET: Tetracycline

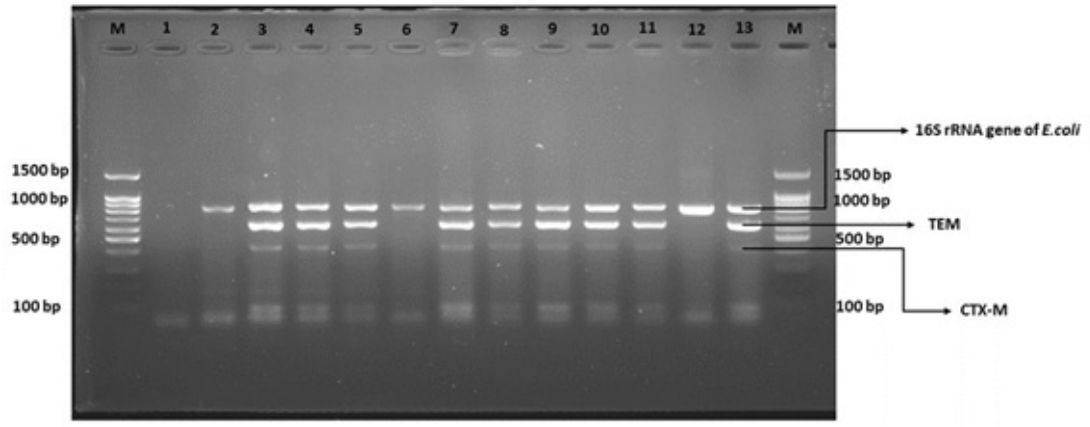

Figure 1 Agarose gel of amplicons generated in the multiplex PCR for ESBL genes detection M: marker 100 bp ladder; Lane 1: negative control; Lane 2: *E. coli* TISTR 527; Lane 3: CNP4; Lane 4: CNP6; Lane 5: KN1; Lane 6: KN2; Lane 7: KN4; Lane 8: KNH1; Lane 9: KNH2; Lane 10: KN3; Lane 11: KNH4; Lane 12: SY301; Lane 13: SY318.

All 11 *E. coli* isolates from broilers contained at least two of the nine genes. The *hlyF* and *iucC* were detected in all APEC isolates, followed by *iroN* and *ompT* (72.72%). Five isolates (45.45%) gave positive results for *iroN ompT hlyF iss iutA tsh iucC,* while only one isolate (KN1) from the liver contained *papC* (Table 3). In addition, the following pattern was uniquely identified: *iroN ompT hlyF iutA tsh iucC* (CNP6) and *iroN ompT hlyF iss iucC* (KN3). Overall, there were five different combination patterns in APEC isolates examined in this study, and none contained all nine genes simultaneously.

Table 3 Determinants of virulence-associated gene profiles and colibacillosis-associated lesions among all 11 isolates

Isolate	Origin	Colibacillosis-associated lesions	Virulence gene profiles
CNP4	Liver	Airsacculitis and fibrinous polyserositis	***iroN*,*ompT*,*hlyF*,*iss*,*iutA*,*tsh*,*iucC***
CNP6	Liver	Airsacculitis	*iroN*,*ompT*,*hlyF*,*iutA*,*tsh*,*iucC*
KN1	Liver	No lesion	*hlyF*,*iucC*,*papC*
KN2	Liver	No lesion	*hlyF*,*iucC*
KN4	Liver	Airsacculitis	***iroN*,*ompT*,*hlyF*,*iss*,*iutA***,*tsh*,*iucC*
KNH1	Heart	Airsacculitis and fibrinous polyserositis	***iroN*,*ompT*,*hlyF*,*iss*,*iutA***,*tsh*,*iucC*
KNH2	Heart	Airsacculitis	***iroN*,*ompT*,*hlyF*,*iss*,*iutA***,*tsh*,*iucC*
KN3	Heart	Airsacculitis	*iroN*,*ompT*,*hlyF*,*iss*,*iucC*
KNH4	Heart	No lesion	*hlyF*,*iucC*
SY301	Liver	Airsacculitis and fibrinous polyserositis	***iroN*,*ompT*,*hlyF*,*iss*,*iutA***,*tsh*,*iucC*
SY318	Liver	Airsacculitis and fibrinous polyserositis	***iroN*,*ompT*,*hlyF*,*iss*,*iutA***,*tsh*,*iucC*

Virulence genes shown in boldface are the five essential genes of APEC strains.

DISCUSSION

E. coli is found in poultry intestines as the normal microflora. However, APEC can spread extra-intestinal and cause colibacillosis outbreaks. Differentiating pathogenic *E. coli* and non-pathogenic *E. coli* is demonstrated by detecting the virulence genes (Won et al., 2009). These include five virulence-characterized genes of APEC; *iroN, ompT, hlyF, iutA,* and *iss*, which have been used for APEC strain markers. In this study, we use the PCR technique to detect the virulence genes. The most prevalent gene in APEC was *hlyF* and *iucC* (100%); *iucC* gene has been regularly found in non-pathogenic *E. coli* (Li et al., 2015). Both *hlyF* and *iucC* were detected in broilers with and without colibacillosis-associated lesions, which indicates that they are not highly related to the APEC pathogenicity. The second majority of APEC (72.72%) isolates were characterized by carrying two genes; *iroN* and *ompT*. These two genes were harbored only in the APEC isolate with colibacillosis-associated lesions, suggesting they were implicated in colibacillosis pathogenesis. The results obtained in the present study supported the previously reported findings that APEC strains from birds with colibacillosis-associated lesions were positive for at least one of 5 virulence-associated genes (Johnson et al., 2008). The current study also detected the *iss, iutA,* and *tsh* genes in 63.63% of APEC isolates. Previous reports suggested many APEC commonly possess those virulence factors (Adhesins: *tsh*, *papC*; Iron acquisition systems: *iutA*, *iucC*, *iroN*; Protectins: *iss*, *ompT*; Toxins: *hlyF*) (Kim et al., 2020). Four virulence factors have been reported with high rates in APEC isolates worldwide; *iroN* (84%) and *iss* (87%) in Canada and *iroN, iss ompT,* and *hlyF* (100%) in Napal (Subedi et al., 2018; Varga et al., 2018). This study isolated all APEC containing five virulence-associated genes from colisepticemia-infected broilers. These results were similar to a study in Egypt (Hussein et al., 2013). In Pakistan, 29.3% of the APEC strains isolated from colibacillosis-affected broilers showed a set of four virulence genes; *iss, tsh, iroN,* and *iutA* (Azam et al., 2019). The results

revealed that virulence gene prevalence differed from those reported. For that reason, it can be summarized that the frequency of the virulence genes may vary in different regions. In addition, an APEC isolate (KN1) contained *papC* in this study, which isolate from a broiler without colibacillosis-associated lesion. Surprisingly, this gene has been reported mainly on human uropathogenic *E. coli* and occasionally on a minority of APEC (Janben et al., 2001). Antibiotics have been widely used to decrease economic losses caused by colibacillosis, so antibiotic resistance among APEC has become a great concern. All APEC isolates from antibiotics-used and antibiotics-free farms exhibited MDR phenotypes in this study. In Thailand, beta-lactam and tetracycline antibiotics are commonly used for prophylaxis in the broiler industry. Unsurprising, all APEC isolates in this study were positive ESBL-production. Similar results showed a high resistance rate to ampicillin (38.9%) and tetracycline (77.8%) from *E. coli* isolated from chickens in northern Thailand (Hanson et al., 2002). In the present study, the highest resistance (100%) were chloramphenicol, erythromycin, and sulfamethoxazole-trimethoprim. Although erythromycin and sulfamethoxazole are frequently used to treat poultry diseases, they were not mentioned to be used in broiler farms in the current study. Therefore, the exposure and cumulation of antibiotics to bacteria encourage MDR challenges (Lay et al., 2021). In this study, the isolates were highly resistant to erythromycin, consistent with previous reports in 2011 (Chansiripornchai et al., 2011). Chloramphenicol is prohibited in livestock; however, resistance gene transfer of this antibiotic has been reported in a conjugation experiment (Nuangmek et al., 2018). The APEC revealed higher and similar resistance to amoxicillin (90.90%) and tetracycline (81.81%) than the previous report of amoxicillin (70.24%) and tetracycline (84.52%) (Thomrongsuwannakij et al., 2020). The remarkable finding in the current study was resistance to fluoroquinolone antibiotic of APEC isolates. Ciprofloxacin and Enrofloxacin resistance were found in APEC isolated from both antibiotics-used and antibiotic-free farms, although these drugs are restricted in food-producing animals in Thailand. Similar findings were observed for *E. coli* isolates in China; it has been reported that more than 70% of APEC isolates were resistant to ciprofloxacin (Yang et al., 2004). The occurrence may be attributed to a mutation in quinolone resistance mechanisms or resistance gene transfer (Aberkane et al., 2023). In our study, 27% of the isolates belonged to a single antimicrobial resistance pattern (Ampicillin-Chloramphenicol-Erythromycin-Tetracycline-Polymyxin B-Sulfamethoxazole-trimethoprim). This finding is consistent with a previous broiler study (Trongjit et al., 2016). MDR was found in two APEC isolated from an antibiotic-free farm in this study. APEC was probably transferred by antibiotic resistance genes from other extra-intestinal resistant bacteria (Farooq et al., 2022) or antibiotic-resistant isolates vertically transferred from parent stocks to broiler flocks (Davis et al., 2018). In the present study, 81.81% of ESBL producers carried TEM and CTX-M genes. Although these genes were detected in 2015, they have been reported in chicken carcasses and porks from Asian countries (Athanasakopoulou et al., 2021; Srichumporn et al., 2022). ESBL-encoding gene was not detected in 18.18% of the present study. However, the genotypically negative ESBL but ESBL-production positive, the reason could be the multiplex PCR performed and did not include all containing resistance genes. Other published studies reported AmpC gene

could be detected in phenotypically positive ESBL-producing *E. coli* (Lee et al., 2019; Seo, 2023). Interestingly, 83.33% of APEC containing five virulence-associated genes were detected TEM and CTX-M genes in the present study. These results were similar to observations in other studies in China (75%) (Li et al., 2015), as well as a study in Spain (77.77%) (Sola-Gines et al., 2015). It is well known that chicken meat has been described as a reservoir of human antibiotic-resistant *E. coli* (Thorsteinsdottir et al., 2010). Further studies will be needed to examine the association between the virulence genes and the antibiotic resistance of APEC strains.

CONCLUSIONS

Our current study characterized virulence-associated genes and ESBL-production of APEC isolated from broilers with and without colibacillosis-associated lesions in eastern Thailand. The results showed no specific virulence gene pattern had been demonstrated for colibacillosis development in broilers. The tested APEC revealed a high prevalence of ESBL-producing *E. coli* isolates in this study. We also studied the distribution of ESBL genes and MDR profiles. The high resistance level observed unmatched the current use of antibiotics on broiler farms in this area. Antibiotic-free broiler farm has shown positive for MDR, probably due to the genetic transfer by antibiotic-resistant bacteria exposed to the chickens. The outcome of this study suggested that *E. coli* could transfer antibiotic-resistance genes to humans through chicken meat consumption. Moreover, continuous monitoring of ESBL-*E. coli* is recommended to track resistance genes transmission through the food chain.

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

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