Molecular and phenotypic characterization of avian pathogenic *Escherichia coli* isolated from commercial broilers and native chickens

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ABSTRACT Many studies have examined avian pathogenic *Escherichia coli* (**APEC**) from commercial broilers but few have examined isolates from native chickens. This study compared APEC isolates from commercial broilers and native chickens in regard to the phylogenetic group and the phenotypic and genotypic antimicrobial resistance profiles. From 100 suspect colibacillosis cases in both commercial broilers and native chickens, a total of 90 broiler isolates and 42 native chicken isolates were identified as *E. coli* by biochemical tests. Phylogenetic grouping revealed that 90 broiler APEC isolates belonged to A group (5.56%), B1 group (22.22%), B2 group (31.11%), and D group (41.11%). The 42 native chicken APEC isolates belonged to A group (35.71%), B1 group (26.19%), B2 group (30.95%), and D group (7.14%). The difference in the allocation to groups A and D of the 2 isolate types was significant ($P < 0.05$). The APEC broiler isolates had a significantly higher multidrug-resistant (**MDR**) rate (80%) than the native chicken isolates (14.29%) ($P < 0.05$). The APEC broiler isolates demonstrated significantly higher resistance rates than the native chicken isolates for amoxicillin (98.89%; 78.57% respectively), chloramphenicol (42.2%; 9.5%), enrofloxacin (68.9%; 7.1%), gentamicin (11.1%; 0%), nalidixic acid (72.2%; 7.1%), sulfamethoxazole + trimethoprim (45.6%; 2.4%), and tetracycline (88.9%; 76.2%) ($P < 0.05$). The APEC broiler isolates had a significantly higher presence compared with the native chicken isolates of the following resistance genes:- by bla_{TEM} (43.3%; 21.4%, respectively), *cml-A* (34.4%; 2.4%), *tetA* (76.7%; 40.5%), *tetB* (26.7%; 0%), *sul2* (23.3%; 14.3%), and *dhfrI* (13.3%; 0%) ($P < 0.05$). The *qnrB* and *qnrS* genes were detected (12.16%; 72.97% respectively), in the APEC broiler isolates resistant to nalidixic acid and/or enrofloxacin while only *qnrS* genes was detected in all 3 APEC native chicken isolates. Regarding the point mutations of *gyrA* and *parC*, all isolates were positive to gyrA83S, gyrA87D, gyrA87L, gyrA87NY, parC80S and parC80I except that gyrA83S was not present in 20 APEC broiler isolates. Antimicrobial stewardship programs should be targeted at the backyard poultry sector as well as the commercial poultry sector.

Key words: avian pathogenic *Escherichia coli*, antimicrobial resistance, commercial broiler, native chicken, resistance gene

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INTRODUCTION

Most *Escherichia coli* are commensal bacteria, but in humans and warm-blooded animals, certain strains are pathogenic. One of the significant forms of pathogenic *E. coli* is extraintestinal pathogenic *E. coli* (**ExPEC**) which may cause systemic infection in humans and animals (Manges et al., 2019). Avian pathogenic *Escherichia coli* (**APEC**), a subpathotype of ExPEC, is the causative agent of colibacillosis in poultry, a disease which can cause significant economic losses due to high morbidity and mortality, medication costs, and condemnation of carcass (Cummins et al., 2019; Kim et al., 2020). Typical colibacillosis signs are airsacculitis, pericarditis, perihepatitis, cellulitis, omphalitis, coligranuloma, and systemic infections (Nolan et al., 2013).

Treatment of the infection of APEC in chickens usually involves antimicrobial chemotherapy. The determination of which antimicrobial to use is based on the in vitro susceptibility of the organism by using the Kirby-Bauer disc diffusion method, the drug pharmacokinetics (the time course of drug absorption, distribution, metabolism, and excretion) and also the clinical efficacy (the

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capacity to produce an effect against bacterial growth) (Luo et al., 2019). Consequently, veterinarians have a limited choice of antimicrobials to use in the poultry industry. In addition, the imprudent use of antimicrobials such as the constant misuse or use as a prophylaxis can lead to an increasing rate of antimicrobial resistance (Weledji et al., 2017). In both human medicine and the poultry industry, antimicrobial resistance (**AMR**) is a significant concern. Several studies have reported that there are genetic similarities between ExPEC in humans and APEC in broilers (Cunha et al., 2017; Sarowska et al., 2019). It has been suggested that there may have been an exchange of transferable mobile genetic elements between pathogenic *E. coli* isolates from animals and humans (Johnson et al., 2008; Johnson et al., 2012). This potential link increases the importance of surveillance of AMR in broiler chickens. In Thailand, people also consume native chickens which are an important part of the traditional agricultural practices of Thai farmers. Traditionally, Thai native chickens are raised by backyard farmers who are likely to have a lower use of antimicrobials compared to commercial broilers raised in the commercial farm. Thai native chicken breeds included in this study were breeds such as Dang and Betong, while commercial breeds included in this study were Cobb and Ross.

There are multiple studies on the antimicrobial resistance profiles of *E. coli* from commercial broilers in Thailand. However, there has been little focus on studies in native chickens. Consequently, the aims of this study were to compare the APEC isolated from commercial broilers and native chickens in regard to: 1) the phylogenetic group; 2) the phenotypic antimicrobial resistance profiles, and 3) the genotypic antimicrobial resistance profiles of the isolates.

MATERIALS AND METHODS

Ethical Approval

The guidelines and legislative regulations on the use of animals for scientific purposes of the Walailak University, Nakorn Si Thammarat, Thailand were followed as certified in permit number WU-AEC-016-62.

Study Period and Location

The study was conducted from January 2020 to November 2020. Bacterial culture and identification, phylogenetic group determination, detection of virulence genes, antimicrobial susceptibility test and detection of AMR genes were carried out at Walailak University, Nakorn Si Thammarat, Thailand.

Sample Collection

In this study, 100 suspect colibacillosis cases from commercial broilers and 100 suspect colibacillosis cases from native chickens were examined. Swabs of the liver, lungs, heart and spleen of affected chickens from different poultry farms located in the South of Thailand were collected. The age of the commercial broilers were ranged from 14 to 42 d old, while the age of the native chickens were ranged from 0.5 to 2 yr old. The internal organs that showed lesions typically associated colibacillosis including perihepatitis, pericarditis, splenitis and/or cellulitis were selected for culture. The detailed information of APEC isolates included in the present study is shown in Supplementary Table 1.

E. coli *Isolation and Identification*

All samples from the commercial broilers and native chickens were plated onto 5% sheep blood agar (**SBA**) and MacConkey agar plates and were aerobically incubated at 37°C for 24 to 48 h. Presumptive pink colonies on MacConkey agar were sub-cultured onto eosin methylene blue (**EMB**) agar and were aerobically incubated at 37°C for 24 h. Presumptive colonies, which showed a metallic green sheen on EMB agar, were sub-cultured onto SBA and aerobically incubated at 37°C for 24 h. Biochemical tests including oxidase, indole and triple sugar iron (**TSI**) tests were performed from the incubated SBA. Presumptive *E. coli* isolates were then stored in tryptone soya broth (**TSB**) with 15% glycerol at -80°C for further study. For the purpose of this study, *E. coli* isolates obtained in pure culture from internal organs showing the typical lesions of colibacillosis were defined as APEC. All media were obtained from OXOID (Basingstoke, Hampshire, England).

DNA Extraction

The *E. coli* isolates were inoculated onto SBA at 37°C for 24 h. Genomic DNA was extracted from fresh cultures by using a PrestoTM mini gDNA bacterial kit (Geneaid Biotech, New Taipei City, Taiwan), in accordance with the manufacturer's instructions as previously described (Thomrongsuwannakij et al., 2017).

Phylogenetic Group Determination With PCR

According to the method of Clermont et al. (2000), the *E. coli* isolates were assigned to the A, B1, B2 or D phylogenetic groups. Primer sequences, gene targets, amplicon lengths, and annealing temperatures are shown in Table 1. Positive samples of each gene were confirmed by sequencing using the relevant PCR primers, after which these DNA preparations were used as positive controls for PCR. Electrophoresis was performed through a 1.5% agarose gel. The PCR products were visualized by ultraviolet transilluminator.

Antimicrobial Susceptibility Test

The APEC isolates were subjected to the Kirby-Bauer disc diffusion method to determine their antimicrobial susceptibility using Muller Hinton agar (**MHA**) (OXOID). The 10 tested antimicrobial agents and their

Table 1. Primers used for the amplification of phylogenetic grouping and antimicrobial resistance genes.

Group	Gene name	Primer sequence	Amplicon size (bp)	Annealing temperature (°C)	Reference
Phylogenetic group	*ChuA*	F: 5′-GACGAACCAACGGTCAGGAT-3′ R: 5′-TGCCGCCAGTACCAAAGACA-3′	279	55	(Clermont et al., 2000)
	YjaA	F: 5′-TGAAGTGTCAGGAGACGCTG-3′ R: 5′-ATGGAGAATGCGTTCCTCAAC-3′	211	55	(Clermont et al., 2000)
	TspE4C2	F: 5′-GAGTAATGTCGGGGCATTCA-3′ R: 5′-CGCGCCAACAAAGTATTACG-3′	152	55	(Clermont et al., 2000)
Antimicrobial groups					
Beta-lactams	*blaTEM*	F: 5′-GAGTATTCAACATTTTCGT-3′ R: 5′-ACCAATGCTTAATCAGTGA-3′	857	58	(Van et al., 2008)
	blaSHV	F: 5′-TCGCCTGTGTATTATCTCCC-3′ R: 5′-CGCAGATAAATCACCACAATG-3′	768	58	(Van et al., 2008)
Phenicols	*cat1*	F: 5′-AGTTGCTCAATGTACCTATAACC-3′ R: 5′-TTGTAATTCATTAAGCATTCTGCC-3′	547	58	(Van et al., 2008)
	cml-A	F: 5′-CCGCCACGGTGTTGTTGTTATC-3′ R: 5′-CACCTTGCCTGCCCATCATTAG-3′	698	58	(Van et al., 2008)
Tetracyclines	*tetA*	F: 5′-GTAATTCTGAGCACTGTCGC-3′ R: 5′-CTGCCTGGACAACATTGCTT-3′	956	57	(Sengeløv et al., 2003)
	tetB	F: 5′-CTCAGTATTCCAAGCCTTTG-3′ R: 5′-ACTCCCCTGAGCTTGAGGGG-3′	414	52	(Sengeløv et al., 2003)
	tetC	F: 5′-CCTCTTGCGGGATATCGTCC-3′ R: 5′-GGTTGAAGGCTCTCAAGGGC-3′	505	65	(Sengeløv et al., 2003)
Trimethoprim	*dhfrV*	F: 5′-AAGAATGGAGTTATCGGGAATG-3′ R: 5′-GGGTAAAAACTGGCCTAAAATTG-3′	391	58	(Van et al., 2008)
Sulfonamides	*sul1*	F: 5′-CTTCGATGAGAGCCGGCGGC-3′ R: 5′-GCAAGGCGGAAACCCGCGCC-3′	433	58	(Sandvang et al., 1998)
	sul2	F: 5′-CGGCATCGTCAACATAACC-3′ R: 5′-GTGTGCGGATGAAGTCAG-3′	720	58	(Maynard et al., 2003)
Quinolones	*qnrA*	F: 5′-TCAGCAAGAGGATTTCTCA-3′ R: 5′-GGCAGCACTATTACTCCCA-3′	657	48	(Wang et al., 2004)
	qnrB	F: 5′-GATCGTGAAAGCCAGAAAGG-3′ R: 5′-ACGATGCCTGGTAGTTGTCC-3′	469	53	(Gay et al., 2006)
	qnrS	F: 5′-ACGACATTCGTCAACTGCAA-3′ R: 5′-TAAATTGGCACCCTGTAGGC-3′	417	53	(Gay et al., 2006)
	gryA83S-Esh	F: 5′-TGGTGACGTAATCGGTAAATACCA-3′ R: 5′-CCGAAGTTACCCTGACCGTCT-3′	80–150	60	(Pholwat et al., 2019)
	gryA83L-Esh	F: 5′-TGGTGACGTAATCGGTAAATACCA-3′ R: 5′-CCGAAGTTACCCTGACCGTCT-3′	80–150	60	(Pholwat et al., 2019)
	gryA87D-Esh	F: 5′-TGGTGACGTAATCGGTAAATACCA-3′ R: 5′-CCGAAGTTACCCTGACCGTCT-3′	80–150	60	(Pholwat et al., 2019)
	gryA87NY-Esh	F: 5′-TGGTGACGTAATCGGTAAATACCA R: 5′-CCGAAGTTACCCTGACCGTCT-3′	80–150	60	(Pholwat et al., 2019)
	parC80I-Esh	F: 5′-CTGAACTGGGCCTGAATGC-3′ R: 5′-ATTGCCGCGAACGATTTC-3′	80–150	60	(Pholwat et al., 2019)
	parC80S-Esh	F: 5′-CTGAACTGGGCCTGAATGC-3′ R: 5′-ATTGCCGCGAACGATTTC-3′	80–150	60	(Pholwat et al., 2019)

corresponding concentrations were as follows: amoxycillin (**AML**) 10 μg/disk, cefotaxime (**CTX**) 30 μg/disk, chloramphenicol (**CHL**) 30 μg/disk, colistin (**CT**) 10 μg/disk, enrofloxacin (**ENR**) 5 μg/disk, gentatmicin (**GEN**) 10 μg/disk, meropenem (**MEM**) 10 μg/disk, nalidixic acid (**NA**) 30 μg/disk, sulfamethoxazole + trimethoprim (19:1) 25 μg/disk, and tetracycline (**TE**) 30 μg/disk. According to the guidelines of the Clinical and Laboratory Standards Institute (**CLSI**) (CLSI, 2013), the Muller Hinton agar (MHA) plates were inoculated with an *E. coli* suspension adjusted to 0.5 McFarland standard. The inoculated plates were incubated for 16 to 18 h at 37°C under aerobic condition (CLSI, 2013). The interpretive criteria used were those recommended for *Enterobacteriaceae* according to the CLSI standards (CLSI, 2015). Resistance to at least 3 classes of antimicrobial agents was considered as MDR. The control organism was *E. coli* ATCC 25922. All antimicrobials were obtained from Sigma-Aldrich (St Louis, MO).

Detection of Antimicrobial Resistance Genes by PCR

Protocols for detecting antimicrobial resistance gene followed the previous studies cited in Table 1. Electrophoresis was performed through a 1.5% agarose gel. The PCR products were visualized by ultraviolet transilluminator.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Science for Windows version 22 (IBM, New York City, NY). The Pearson's chi-square test with statistical significance set at level $P < 0.05$ was used to investigate the relationship of resistance rate and resistance genes between APEC isolated from commercial broilers and APEC isolated from native chickens. In addition, the Pearson's chi-square test was also used to investigate the relationship among the phylogenetic group of APEC isolated from commercial broilers and native chickens.

Table 2. Phylogenetic group of avian pathogenic *Escherichia coli* (APEC) isolated from commercial broilers and native chickens.

Phylogenetic group	% of APEC broiler isolates (n = 90)	% of APEC native chicken isolates (n = 42)
A	5.6[1]	35.7[1]
B1	22.2	26.2
B2	31.1	30.9
D	41.1[1]	7.1[1]

[1]There were significant differences ($P < 0.05$) in groups A and D between both APEC groups.

RESULTS

APEC Isolates

In this study, 100 suspect colibacillosis cases from commercial broilers and 100 suspect colibacillosis cases from native chickens were cultured and 90 and 42 *E. coli* isolates (respectively) were obtained. A single isolate obtained from a pure culture of an internal organ was selected as the representative isolate for each bird. As per the study definition, these pure cultures of *E. coli* were defined as APEC. These isolates were presumptively identified *as E. coli* by using biochemical tests and were then further examined for additional phenotypic and genotypic characteristics as detailed below.

Phylogenetic Group

Phylogenetic grouping demonstrated that the 90 APEC isolates from commercial broilers belong to D group (41.1%), B2 group (31.1%), B1 group (22.2%), and A group (5.6%). On the other hand, 42 strains of APEC isolated from native chickens belong to A group (35.7%), B2 group (30.9%), B1 group (26.2%), and D group (7.1%) (Table 2). There were significant differences ($P < 0.05$) in groups A and D between both 2 APEC groups.

Phenotypic Antimicrobial Resistance

The quality control strain, *E. coli* ATCC 25922, always gave results within the required range. Overall, the Thai APEC isolates from commercial broilers were more resistant to various classes of antimicrobial agents compared to the Thai APEC strains isolated from native chickens, except CTX. The MDR rate, defined as resistance to 3 or more antimicrobial classes, of the Thai APEC isolates from commercial broilers was 80% while the Thai APEC isolates from native chickens was only 14.3%. The Thai APEC broiler isolates demonstrated a high resistance rate to AMX (98.9%), TET (88.9%), NAL (72.2%), ENR (68.9%), SXT (45.6%), and CHL (42.2%). In contrast, the Thai APEC native chicken isolates showed a high resistance rate only to AMX (78.6%) and TET (76.2%) (Figure 1). In this study, all of the Thai APEC broiler isolates were sensitive to CT and the Thai APEC native chicken isolates |were totally sensitive to GEN and MEM. Significant differences ($P < 0.05$) between 2 APEC groups were found in resistance to AMX, CHL, ENR, GEN, NAL, SXT, TET, and in the occurrence of MDR. The most common resistance patterns found in this study were AML-ENR-NAL-SXT-TET (17.8%) for the APEC broiler isolates and AML-TET (59.5%) for the APEC native chicken isolates (Table 3).

AMR Genes

The genes responsible for a diversity of the AMR characteristics seen in the phenotypic testing were investigated by multiplex PCR assays. Regarding the APEC broiler isolates, the resistance genes detected in 20% or more of the isolates were *tetA* (76.7%), bla_{TEM} (43.3%), *cmlA* (34.4%), *sul1* (27.8%), *tetB* (26.7%) and *sul2* (23.3%) whereas in the APEC native chicken isolates the only resistance genes detected in 20% or more of the isolates were *tetA* (40.5%) and bla_{TEM} (21.4%). Significant differences ($P < 0.05$) between 2 APEC groups were found for the presence of bla_{TEM}, *cmlA, tetA, tetB, sul2,* and *dhfrI.* The results showed good correlation between resistance phenotype and genotypes in these APEC isolates (Figure 1 and Table 4).

Screening for qnr Genes and gyrA and parC Point Mutations

Three Thai APEC native chicken isolates which were resistant to nalidixic acid and/or enrofloxacin were positive to *qnrS* gene (100%) and all *gyrA* and *parC* point mutations were identified in this study. In addition, 74 of the Thai APEC broiler isolates which were resistant to nalidixic acid and/or enrofloxacin were positive to *qnrB* (12.1%) and *qnrS* (73%) and were positive to gyrA83S (97.3%), gyrA87D (100%), gyrA87L (100%), gyrA87NY (100%), parC80S (100%), and parC80I (100%) (Table 5.).

DISCUSSION

While *E. coli* is a part of the normal flora of the intestinal tract of poultry, specific strains known as APEC, a subgroup of ExPEC, have special virulence factors and can cause avian colibacillosis (Kathayat et al., 2021). This disease is a major problem for the commercial broiler industry (Younis et al., 2017; Kim et al., 2020; Thomrongsuwannakij et al., 2020) but has been little studied in native chickens. This study provides the results of 1) the phylogenetic group; 2) the phenotypic antimicrobial resistance profiles, and 3) the genotypic antimicrobial resistance profiles of APEC isolated from commercial broilers and native chickens.

For the purpose of this study, *E. coli* isolates that were obtained in pure culture from an internal organ showing the typical lesions of colibacillosis were defined as APEC. A total of 90 and 42 APEC isolates from commercial broilers and native chickens were characterized by both phenotype and genotype characteristics. Based

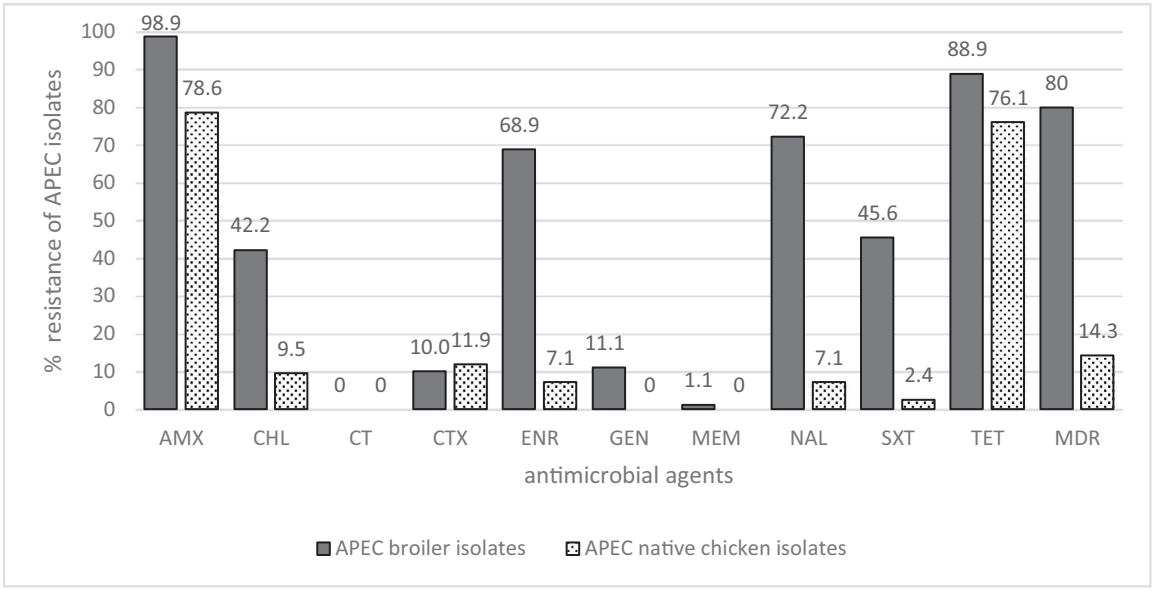

Figure 1. The frequency of resistance to 10 antimicrobial agents in the avian pathogenic *Escherichia coli* (APEC) isolates from commercial broilers (n = 90) and native chickens (n = 42). Abbreviations: AMX, amoxicillin, CHL, chloramphenicol; CT, colistin; CTX, cefotaxime; ENR, enrofloxacin; GEN, gentamicin; MDR, multidrug resistance; MEM, meropenem; NAL, nalidixic acid; SXT; trimethoprim-sulfamethoxazole; TET, tetracycline. Significant differences ($P < 0.05$) between 2 APEC groups were found for resistance to AMX, CHL, ENR, GEN, NAL, SXT, TET, and for the occurrence of MDR.

Table 3. Antimicrobial resistance pattern of avian *pathogenic Escherichia coli* (APEC) isolates from commercial broilers (n = 90) and native chickens (n = 42).

Source	Antimicrobial resistance pattern	No. of isolates (%)
APEC broiler isolates	AML-ENR-NAL-SXT-TET	16 (17.8)
	AML-ENR-NAL-TET	14 (15.6)
	AML-CHL-ENR-NA-SXT-TET	12 (13.3)
APEC native chicken isolates	AML-TET	25 (59.5)

Notes: Only the antimicrobial resistance patterns represented by at least 5 isolates are shown.

Abbreviations: AMX, amoxicillin; CHL, chloramphenicol; ENR, enrofloxacin; NAL, nalidixic acid; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline.

Table 4. Antimicrobial resistance genes found in avian pathogenic *Escherichia coli* (APEC) isolates from broilers and native chickens.

Antimicrobial resistance gene[1]	% of APEC broiler isolates (n = 90) with indicated resistance gene	% of APEC native chicken isolates with indicated resistance gene (n = 42)
Beta-lactam		
bla$_{TEM}$	43.3	21.4
bla$_{SHV}$	1.1	7.1
Chloramphenicol		
cat1	3.3	0
cmlA	34.4	2.4
Tetracycline		
tetA	76.7	40.5
tetB	26.7	0
tetC	1.1	0
Sulfonamide		
sul1	27.8	19.0
sul2	23.3	14.3
Trimethoprim		
dhfrI	13.3	0

[1]Significant differences ($P < 0.05$) between 2 APEC groups were found in *bla*$_{TEM}$, *cmlA*, *tetA*, *tetB*, *sul2*, and *dhfrI*.

on the results of this study, the majority of the APEC isolates from the commercial broilers belonged to Clermont phylogenetic groups D (41.1%) and B2 (31.1%). In contrast, the majority of the APEC isolates from native chickens belonged to Clermont phylogenetic groups A (35.7%) and B2 (30.9%). Clermont et al. (2000) reported that virulent ExPEC mainly belong to groups B2 and D meaning that the APEC strains isolated from native chickens are different from the APEC strains isolated from commercial broilers.

AMR is a serious concern in both human medicine and the poultry industry (Younis et al., 2017; Sarowska et al., 2019). It is accepted that there are genetic similarities between ExPEC in humans and APEC in broilers (Cunha et al., 2017; Sarowska et al., 2019). This has resulted in the suggestion that there may have been an exchange of transferable mobile genetic elements between pathogenic *E. coli* isolates from animals and humans (Johnson et al., 2008; Johnson et al., 2012).

Based on the phenotypic results, the Thai APEC broiler isolates were significantly more resistant to almost all the classes of antimicrobial agents tested as compared to the Thai APEC native chicken isolates. The exception was that CTX resistance in both groups did not significantly differ (10% in the APEC broiler isolates and 11.9% in the APEC native chicken isolates). This suggests that many antibiotics are not used as frequently in native chickens as in broilers and that CTX is not used commonly for treatment of colibacillosis in either bird type. In addition, the MDR rate of the Thai APEC broiler isolates was 80% and the most common resistance pattern found in this group was AML-ENR-NAL-SXT-TET (17.8%) which is in accordance with the fact that the drug of choice for the treatment of colibacillosis in Thailand is typically a drug in groups of

Table 5. Occurrence of *qnr* genes and point mutations in *gyrA* and *parC* genes in avian pathogenic *Escherichia coli* (APEC) isolates resistant to nalidixic acid or enrofloxacin.

Source	% of the isolates found with indicated genes or indicated point mutations in *gyrA* and *parC*								
	qnrA	*qnrB*	*qnrS*	*gyrA83S*	*gyrA87D*	*gyrA87L*	*gyrA87NY*	*parC80S*	*parC80I*
APEC broiler isolates which were resistant to either NAL or ENR (n = 74)	0	12.16	72.97	97.3	100	100	100	100	100
APEC native chicken isolates which were resistant to either NAL or ENR (n = 3)	0	0	100	100	100	100	100	100	100

beta-lactams, tetracyclines or sulfonamides (Thomrongsuwannakij et al., 2020). Kim and colleagues (Kim et al., 2020) also reported that the APEC isolates from broiler chickens demonstrated high resistance to ampicillin (AMP) (83.5%), NAL (65.8%), TET (64.6%), and ciprofloxacin (CIP) (46.8%). On the other hand, the MDR rate of the Thai APEC native chicken isolates was just 14.3% and the most common resistance pattern in this group was AML-TET (59.5%).

Native chickens are typically reared as free-living birds that range outdoors and there is a low use of antimicrobials (Chalermchaikit et al., 2005). The farmers use only a limited range of antimicrobials (mainly penicillin, amoxicillin or tetracycline) for treatment of bacterial infections (Chalermchaikit et al., 2005). Interestingly, the resistance rate against NAL (72.2%), ENR (68.9%) and SXT (45.6%) in the APEC broiler isolates was significantly higher compared to the native chicken group. This suggests that these drugs are being routinely used in the treatment of commercial broilers but not in native chickens.

In this study, 2 β-lactamases genes were identified (bla_{TEM} and bla_{SHV}) with bla_{TEM} being the most prevalent in both groups of *E. coli* which is in an agreement with a recent study in commercial broilers (Younis et al., 2017). This suggests that bla_{TEM} is widespread in poultry isolates of *E. coli*. A previous study conducted in Korea reported that the bla_{TEM} only codes for narrow-spectrum β-lactamases that can inactivate penicillins and aminopenicillins (Seo and Lee, 2019). This is consistent with our results as we found a high resistance to AMX and low resistance rates for CTX, a cephalosporin, and MEM, a carbapenem, even in the presence of bla_{TEM}.

In this study, the *tetA* gene was the most predominant resistance gene found in the APEC isolates from commercial broilers (76.7%) and from native chickens (40.5%) while *tetB* was found only the APEC isolates from commercial broilers (26.7%). The *tetA* and *tetB* genes encode efflux mechanisms and are the most common TET resistance determinant in *E. coli.* (Van et al., 2008). Sulfonamide resistance is conferred by *sul1* and *sul2* (Razavi et al., 2017). In this study, the *sul1* and *sul2* gene were detected at similar percentage in each group which is in contrast with a previous study which found that the *sul2* gene was present in a major proportion of the isolates (Kim et al., 2020). CHL resistance is mediated by enzymatically by the plasmid-located CHL acetyltransferase gene *catA1* and nonenzymatically by the CHL resistance gene *cmlA* (White et al., 2000). In this study, *cmlA* (34.44%) was found in a larger proportion compared to *catA1* (3.33%).

The APEC isolates in this study were resistant to fluoroquinolones, antimicrobials critically important to human medicine, identified by the presence of PMQR genes (*qnrA, qnrB* and *qnrS*) and the mutations of QRDR of *gyrA* and *parC* (Yoon et al., 2020). In this study, 74 Thai APEC isolates from commercial chickens which were resistant to either nalidixic acid or enrofloxacin were positive to *qnrB* (12.16%) and *qnrS* (72.97%) genes. As well, the 3 Thai APEC native chicken isolates which were resistant to nalidixic acid and/or enrofloxacin were positive to *qnrS* (100%) gene. We detected mutations of QRDR of *gyrA* and *parC* genes in this study by PCR as previously described (Thomrongsuwannakij et al., 2017), obtaining rapid and low cost results compared to a conventional method by using Sanger sequencing and alignment with the NCBI database. We used PCR as it has been reported that fluoroquinolone resistance was mainly associated with mutations in *gyrA* codon positions 83 and 87 and *parC* codon position 80 (Pholwat et al., 2019; Muggeo et al., 2020). The results in this study showed that the point mutations of *gyrA* and *parC* from all samples were positive to gyrA83S, gyrA87D, gyrA87L, gyrA87NY, parC80S, and parC80I, except that gyrA83S was found in only 97.3% of the broiler APEC isolates, meaning that both mechanisms of fluoroquinolone resistance were working together in these APEC isolates. This result is consistent with recent studies of APEC strains from the United States and South Korea (Seo and Lee, 2019; Yoon et al., 2020).

The control of APEC infections is a critical public health concern, particularly as APEC isolates harbor MDR genes. There is also the potential for APEC isolates to transfer these resistance genes to human-specific *E. coli* or other pathogenic bacteria. Antimicrobial stewardship programs should engage the backyard poultry sector as well as the commercial poultry sector. Continuous monitoring to track APEC transmission and the associated AMR profiles in poultry farms is recommended.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2021.101527.

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