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Meat ducks as carriers of antimicrobial-resistant *Escherichia coli* harboring transferable R plasmids

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ABSTRACT

Importance: Antimicrobial resistance (AMR) is a serious public health threat. AMR bacteria and their resistance determinants in food can be transmitted to humans through the food chain and by direct contact and disseminate directly to the environment.
Objective: This study examined the AMR characteristics and transferable R plasmids in *Escherichia coli* isolated from meat ducks raised in an open-house system.
Methods: One hundred seventy-seven (n = 177) commensal *E. coli* were examined for their antimicrobial susceptibilities and horizontal resistance transfer. The plasmids were examined by PCR-based plasmid replicon typing (PBRT) and plasmid multi-locus sequence typing (pMLST).
Results: The highest resistance rate was found against ampicillin (AMP, 83.0%) and tetracycline (TET, 81.9%), and most isolates exhibited multidrug resistance (MDR) (86.4%). The R plasmids were conjugally transferred when TET (n = 4), AMP (n = 3), and chloramphenicol (n = 3) were used as a selective pressure. The three isolates transferred resistance genes either in AMP or TET. The *bla*CTX-M1 gene resided on conjugative plasmids. Five replicon types were identified, of which Inc FrepB was most common in the donors (n = 13, 38.4%) and transconjugants (n = 16, 31.2%). Subtyping F plasmids revealed five distinct replicons combinations, including F47:A-:B- (n = 2), F29:A-:B23 (n = 1), F29:A-:B- (n = 1), F18:A-B:- (n = 1), and F4:A-:B- (n = 1). The chloramphenicol resistance was significantly correlated with the other AMR phenotypes ($p < 0.05$).
Conclusions and Relevance: The meat ducks harbored MDR *E. coli* and played an important role in the environmental dissemination of AMR bacteria and its determinants. This confirms AMR as a health issue, highlighting the need for routine AMR monitoring and surveillance of meat ducks.

Keywords: Antibiotic resistance; ducks; *Escherichia coli*; R plasmids; Thailand

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INTRODUCTION

Antimicrobial resistance (AMR) is a serious public health threat with a greater burden in low- and middle-income countries [1]. The overuse and misuse of antimicrobial agents in food animal production are considered important contributors to the development of AMR [2]. In addition, antibiotics belonging to the same classes are administered to humans, animals, and plants. These place AMR as a One Health problematic issue [3] requiring complex-unified inter-sectoral collaboration.

Duck rearing is an integral part of the poultry industry in many Asian countries (e.g., Malaysia, Vietnam, China, Bangladesh, Indonesia and Thailand) [4]. Duck meat is considered delicious and rich in amino acids and polyunsaturated fatty acids but low in fat. It is also considered an excellent source of protein for human consumption. Hence, it is gaining popularity and gradually displacing chicken meat [5]. Meat duck production in Thailand has increased dramatically, and the country has been placed among the top 10 countries in the world for duck meat production over the last decade, with an annual production of 7,000,000 meat ducks [6]. Traditional and modern integrated systems are used to raise ducks, and rearing ducks in an open-house system is common in the country [7]. This duck-raising practice has raised public health concerns because of insufficient biosecurity measures in animal care and farm management. The concern is exaggerated by the significantly increased use of antimicrobials in the duck industry in recent years in the areas with the intensification of duck production, including Southeast Asia [8], where the regulation of antimicrobial use in animals has been poorly enforced in most countries. In Thailand, the use of antibiotics in livestock is regulated by the Department of Livestock Development. The country has drafted and enacted laws and regulations containing AMR associated with food animals, but none have been specific to poultry. For example, the Notification of the Ministry of Agriculture and Cooperatives that specifically prohibits the use of all antibiotics in animal feed as growth promoters was released in 2015 [9]. The law on "Characteristics and conditions of animal feed containing drugs prohibited from producing, importing, selling and using" launched in 2018 addressed medicated feed containing polymyxin B, cephalosporins, and fluoroquinolones [10]. One year later, the regulation of antimicrobial drugs that must not be mixed in animal feed for prophylactic purposes was announced [11]. Although healthy food animals (including meat ducks) are expected to be slaughtered for human consumption, meat ducks that look healthy could be implicated as carriers of AMR bacteria and determinants that endanger the health of humans, animals and environment in the long run [5].

AMR monitoring and surveillance in bacteria from food animals for public health purposes includes commensal *Escherichia coli* as a Gram-negative representative indicator [12]. This bacterial species serves as a reservoir for AMR determinants that can be transmitted to other bacterial pathogens [13], as demonstrated in previous studies conducted in Laos [14] and Nigeria [15]. Commensal *E. coli* possesses a variety of conjugative R plasmids carrying various AMR genes and play a role in the evolution and spread of AMR. Recognizing the significant role of plasmids in the AMR distribution, plasmid-based tracking has been recommended for inclusion in AMR surveillance programs [16].

Plasmid incompatibility (Inc) group testing is a classical method for identifying and classifying plasmids [17]. Plasmids from the same Inc group can neither coexist in the same bacterial cells nor be transmitted between them because they share the same replication control or partitioning mechanisms. The existence of bacterial strains containing plasmids from the same

Inc group in those from various origins suggests the horizontal transmission of such plasmids with close phylogenetic relationships [18]. For example, members of the Enterobacteriaceae family (e.g., *E. coli*, *Klebsiella pneumoniae*, and *Salmonella enterica*) frequently contain IncF plasmids [19]. IncX plasmids have been identified in *Salmonella* [20] and *E. coli* [21] but are also present in *Pseudomonas* spp*., Acinetobacter* spp., and *Aeromonas* spp. The relationship between particular Inc groups and bacterial species may be due to the capacity of plasmids for stable replication in particular bacterial hosts [22]. Knowledge and understanding of the plasmid variety and transmission will be beneficial in developing a strategic action plan to reduce AMR [13].

AMR studies have been conducted extensively in livestock, especially pigs and broilers. Previous studies reported the AMR extent, distribution, and genetic characteristics, including plasmid replicons in livestock, meat, and humans [23]. IncF was the predominant replicon type in *E. coli* isolated from pigs, pork, and humans [22]. Nevertheless, knowledge is still limited in bacteria of a duck origin. This study examined the AMR characteristics (i.e., antimicrobial susceptibilities and R-plasmid transferability) and transferable plasmids in *E. coli* from meat ducks in Thailand.

METHODS

Sample collection and bacterial isolation

One hundred and seventy-seven commensal *E. coli* isolates were obtained from the bacterial stock of the Microbiology Laboratory, Faculty of Veterinary Medicine, Kasetsart University, Nakhon Pathom. They were isolated from cloacal swabs collected in 2018 and 2019 from three duck farms in Nakhon Pathom province, which has the highest population of meat ducks in Thailand. One farm has around 2,000 ducks, which were fed commercial feed. Approximately 4,000 ducks were in two other farms where the ducks were fed a home-mixed feed formulation. Water for all farms was from an underground source.

Each farm raised ducks using a conventional open-house system and had only one flock. During the rearing period, amoxicillin was the most common antibiotic used to treat sick birds by adding it to the drinking water. The cloacal swabs were collected individually from ducks aged 60–70 days before being sent to the slaughterhouses. *E. coli* was isolated by the direct inoculation method [24,25]. Briefly, the cloacal swab samples were directly inoculated on MacConkey agar (Difco; BD, USA). Typical colonies were streaked on Eosin Methylene Blue (EMB) agar (Difco; BD). The *E. coli* isolates were confirmed biochemically using the indole production test. One isolate was kept from each positive sample as 20% glycerol stock at −80°C. Upon arrival at the authors' laboratory, all *E. coli* were reconfirmed by growing on EMB and MacConkey agar (Difco; BD).

Antimicrobial susceptibility testing and detection of extended-spectrum β-lactamase (ESBL) production

All *E. coli* isolates (n = 177) were examined for their susceptibilities to 15 antimicrobial agents using the broth microdilution method on a Sensititre Automatic Machine (Thermo Scientific, USA). The regionally customized Asia surveillance plates, ASSECAF and ASSECB, were used (TREK Diagnostic Systems, UK). The antimicrobial agents included the following (abbreviations and clinical breakpoints in parentheses): ampicillin (AMP, 32 µg/mL), azithromycin (AZI, 32 µg/mL), cefotaxime (FOT, 4 µg/mL), ceftazidime (TAZ, 16 µg/mL), chloramphenicol (CHL, 32 µg/mL), ciprofloxacin (CIP, 4 µg/mL), colistin (COL, 4 µg/mL),

gentamicin (GEN, 16 µg/mL), meropenem (MERO, 4 µg/mL), nalidixic acid (NAL, 32 µg/mL), streptomycin (STR, 16 µg/mL), tetracycline (TET, 16 µg/mL), tigecycline (TGC, 1 µg/mL), trimethoprim (TMP, 16 µg/mL), and sulfamethoxazole (SMX, 512 µg/mL) [26]. The reference strains *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213 were used as the quality-control strains. Multidrug resistance (MDR) is defined as a strain resistant to at least three antimicrobials in different classes.

The initial AST, *E. coli* isolates (n = 10) resistant to FOT and TAZ were then analyzed to confirm ESBL production using the Sensititre EUVSEC2 plate. ESBL production was confirmed based on the minimum inhibitory concentration (MIC) results for FOT, TAZ, FOT/clavulanate, and TAZ/clavulanate [26]. An ESBL-producer was defined as an isolate showing an at least a two to three-fold decrease in concentration in an MIC for either FOT or TAZ tested in combination with clavulanic acid versus its MIC when tested alone.

Conjugation experiment

The horizontal transfer of R plasmids was examined using the biparental mating technique [23]. The MDR *E. coli* isolates (n = 148) served as donors using AMP/TET (n = 77), AMP/TET/COL (n = 15), AMP/TET/CHL (n = 10), AMP/COL/CHL (n = 1), AMP/TET/CHL/COL (n = 32), AMP (n = 6), TET (n = 6), and COL (n = 1) as selective pressure. Only one antibiotic was used as a selective pressure in each plate. The rifampicin-resistant *Salmonella* Enteritidis (SE12) strains (SE12rif^r, MIC= 256 µg/mL) were used as recipients [23]. Briefly, the donor and recipient overnight cultures were diluted by mixing 80 µL of the culture with 4 mL of fresh Luria Bertani broth (Difco; BD). In a microcentrifuge, the mating of donor and recipient cultures were mated in a 1:1 ratio. The bacterial cells were collected, placed onto 0.45 µm pore size filters on LB agar plates (Millipore; Merck, Germany), and incubated at 37℃ overnight. The conjugation mixture was collected and washed in a 0.9% NaCl solution. The transconjugants were selected on LB agar plates with rifampicin (32 µg/mL) and an appropriate antibiotic, i.e., AMP (150 µg/mL), TET (15 µg/mL), CHL (25 µg/mL), and COL (2 µg/mL), and distributed. The transconjugants were confirmed to be *Salmonella* on Xylose Lysine Deoxycholate agar (Difco; BD). The conjugation rate was quantified by the ratio of the number of transconjugants to the number of donors.

PCR amplification and DNA sequencing

The DNA template from the donor and transconjugants was prepared by whole-cell boiling lysis [27]. **Table 1** lists all primers used in this study. The PCR reactions were performed using a TopTaq PCR Master Mix Kit (QIAGEN, Germany) according to the manufacturer's instructions. The PCR amplicons were purified using Nucleospin Gel and PCR cleanup (Macherey-Nagel, Germany) and submitted to BIONICS Laboratories (Korea) for nucleotide sequencing.

Detection of β-lactamase genes

The presence of β-lactamase genes, including bla_{TEM}, bla_{CTX-M}, and bla_{SHV}, was detected by PCR and confirmed by nucleotide sequencing in the ESBL-producing donor isolates (n = 2) and corresponding transconjugants (n = 4) [28]. The bla_{CTX-M}-positive isolates were further determined for the specific CTX-M subgroups (i.e., groups 1, 2, and 9) using multiplex-PCR [29].

PCR-based plasmid replicon typing (PBRT) and plasmid multi-locus sequence typing (pMLST)

The *E. coli* donors (n = 13) that conjugally transferred plasmids when using AMP (n = 3), TET (n = 4), CHL (n = 3), and AMP/TET (n = 3) as a selective pressure. One of their corresponding

Table 1. Primers used in this study

Target	Primer	Sequence (5′–3′)	Amplicon size (bp)	Reference
PBRT				
IncHI1	HI1-F	GGAGCGATGGATTACTTCAGTAC	471	[30]
	HI1-R	TGCCGTTTCACCTCGTGAGTA		
IncHI2	HI2-F	TTTCTCCTGAGTCACCTGTTAACAC	644	[30]
	HI2-R	GGCTCACTACCGTTGTCATCCT		
IncI1	I1-F	CGAAAGCCGGACGGCAGAA	139	[30]
	I1-R	TCGTCGTTCCGCCAAGTTCGT		
IncX	X-F	AACCTTAGAGGCTATTTAAG TTGCTGAT	376	[30]
	X-R	TGAGAGTCAATTTTTATCTCATGTTTTAGC		
IncL/M	L/M-F	GGATGAAAACTATCAGCATCTGAAG	785	[30]
	L/M-R	CTGCAGGGGCGATTCTTTAGG		
IncN	N-F	GTCTAACGAGCTTACCGAAG	559	[30]
	N-R	GTTTCAACTCTGCCAAGTTC		
IncFIA	FIA-F	CCATGCTGGTTCTAGAGAAGGTG	462	[30]
	FIA-R	GTATATCCTTACTGGCTTCCGCAG		
IncFIB	FIB-F	GGAGTTCTGACACACGATTTTCTG	702	[30]
	FIB-R	CTCCCGTCGCTTCAGGGCATT		
IncW	W-F	CCTAAGAACAACAAAGCCCCCG	242	[30]
	W-R	GGTGCGCGGCATAGAACCGT		
IncY	Y-F	AATTCAAACAACACTGTGCAGCCTG	765	[30]
	Y-R	GCGAGAATGGACGATTACAAAACTTT		
IncP	P-F	CTATGGCCCTGCAAACGCGCCAGAAA	634	[30]
	P-R	TCACGCGCCAGGGCGCAGCC		
IncFIC	FIC-F	GTGAACTGGCAGATGAGGAAGG	262	[30]
	FIC-R	TTCTCCTCGTCGCCAAACTAGAT		
IncA/C	A/C-F	GAGAACCAAAGACAAAGACCTGGA	465	[30]
	A/C-R	ACGACAAACCTGAATTGCCTCCTT		
IncT	T-F	TTGGCCTGTTTGTGCCTAAACCAT	750	[30]
	T-R	CGTTGATTACACTTAGCTTTGGAC		
IncFIIA	FIIs-F	CTGTCGTAAGCTGATGGC	270	[30]
	FIIs-R	CTCTGCCACAAACTTCAGC		
IncF	F-F	TGATCGTTTAAGGAATTTTG	270	[30]
	F-R	GAAGATCAGTCACACCATCC		
IncK	K-F	GCGGTCCGGAAAGCCAGAAAC	160	[30]
	K-R	TCTTTCACGAGCCCGCCAAA		
IncB/O	B/O-F	GCGGTCCGGAAAGCCAGAAAAC	159	[30]
	B/O-R	TCTGCGTTCCGCCAAGTTCGA		
IncF-RST				
FII	FII-F	CTGATCGTTTAAGGAATTTTT	258–262	[32]
	FII-R	CACACCATCCTGCACTTA		
FIIs	FIIS-F	CTAAAGAATTTTGATGGCTGGC	259–260	[32]
	FIIS-R	CAGTCACTTCTGCCTGCAC		
FIA	FIA-F	CCATGCTGGTTCTAGAGAAGGTG	462	[32]
	FIA-R	GTATATCCTTACTGGCTTCCGCAG		
FIB	FIBs-F	TGCTTTTATTCTTAAACTATCCAC	683	[32]
	FIB-R	CTCCCGTCGCTTCAGGGCATT		
ESBLs				
*bla*TEM	blaTEM-F	GCGGAACCCCTATTT	964	[28]
	blaTEM-R	TCTAAAGTATATATGAGTA AACTTGGTCT		
*bla*SHV	blaSHV-F	TTCGCCTGTGTATTATCTCCCTG	854	[28]
	blaSHV-R	TTAGCGTTGCCAGTGYTG		
*bla*CTX-M	blaCTX-M-F	CGATGTGCAGTACCAGTAA	585	[47]
	blaCTX-M-R	AGTGACCAGAATCAGCGG		
CTX-M Gr. 1	Mul-CTXMG1-F	TTAGGAARTGTGCCGCTGYA	688	[29]
	Mul-CTXMG1-R	CGATATCGTTGGTGGTRCCAT		
CTX-M Gr. 2	Mul-CTXMG2-F	CGTTAACGGCACGATGAC	404	[29]
	Mul-CTXMG2-R	CGATATCGTTGGTGGTRCCAT		
CTX-M Gr. 9	Mul-CTXMG9-F	TCAAGCCTGCCGATCTGGT	561	[29]
	Mul-CTXMG9-R	TGATTCTCGCCGCTGAAG		

PBRT, PCR-based plasmid replicon typing; RST, replicon sequence typing; ESBL, extended-spectrum β-lactamase.

transconjugants (n = 16) was selected for PBRT. Screening of 18 Inc groups of plasmids was conducted using five multiplex PCRs (i.e., HI1/HI2/I1-Iγ, X/L-M/N, FIA/FIB/W, Y/P/FIC, and A-C/T/FIIs) and three simplex PCRs (i.e., F, K, and B/O) [30].

Based on the PBRT results, six *E. coli* donors (i.e., A144, A183, C248, C249, C250, and C253) that possess IncF replicons were selected for further subtyping according to the pMLST scheme [31]. The FIA, FIB, and FIC were PCR amplified, purified, and submitted for nucleotide sequencing [32]. The Fasta files of individual allele-specific sequences were uploaded to identify the allele number and sequence type (ST) assignment using the pMLST database (https://www.pubmlst.org/plasmid/).

Statistical analysis

Descriptive statistics were used to examine the percentage of AMR using Microsoft Excel. The associations of AMP and TET resistance with other antibiotics were determined using a χ^2 test and calculating the odds ratio (OR) by SPSS program version 22.0 (IBM Corp., USA). A *p* value < 0.05 was considered significant. ORs < 1 and > 1 indicated negative and positive associations, respectively.

RESULTS

Antimicrobial susceptibilities and ESBL production

All *E. coli* isolates were resistant to at least one antimicrobial agent (**Table 2**). The highest percentage resistance was observed against AMP (83.0%) and TET (81.9%), followed by STR (75.7%), TGC (72.8%), and SMX (60.4%), as shown in (**Fig. 1**). Only 2.25% of the *E. coli* isolates were resistant to CIP. The resistance rates to COL and CHL were 27.6% and 24.2%, respectively. None of the isolates were resistant to MERO. Ten out of 177 *E. coli* isolates (5.6%) were resistant to FOT and TAZ. Nine isolates (5.1%) were confirmed to be ESBL producers. MDR was observed in 86.4% of the *E. coli* isolates (n = 153). Seventy-five resistance patterns were identified, of which the most prevalent resistance patterns were AMP-STR-TET-TGC (6.21%), AMP-STR-TET (5.64%), and AMP-TET-TGC (5.64%) (**Table 2**).

Table 2. AMR pattern of *Escherichia coli* isolated from meat ducks (n = 177)

AMR pattern[a]	Number of isolates (%)
AMP-TET	4 (2.25)
STR-SMX	4 (2.25)
AMP-STR-TET	10 (5.64)
AMP-TET-TGC	10 (5.64)
STR-SMX-TGC	5 (2.82)
AMP-STR-TET-TGC	11 (6.21)
AMP-AZI-STR-TET-TGC	6 (3.38)
AMP-STR-TET-TGC-TMP	5 (2.82)
AMP-STR-SMX-TET-TGC-TMP	8 (4.51)
AMP-COL-STR-SMX-TET-TGC-TMP	4 (2.25)
AMP-CHL-COL-STR-SMX-TET-TGC-TMP	7 (3.38)
AMP-CHL-COL-NAL-STR-SMX-TET-TGC-TMP	5 (2.82)

AMR, antimicrobial resistance; AMP, ampicillin; TET, tetracycline; STR, Streptomycin; SMX, sulfamethoxazole; TGC, Tigecycline; AZI, azithromycin; TMP, trimethoprim; COL, colistin; CHL, chloramphenicol; NAL, Nalidixic acid.
[a]The AMR pattern with at least four isolates are shown.
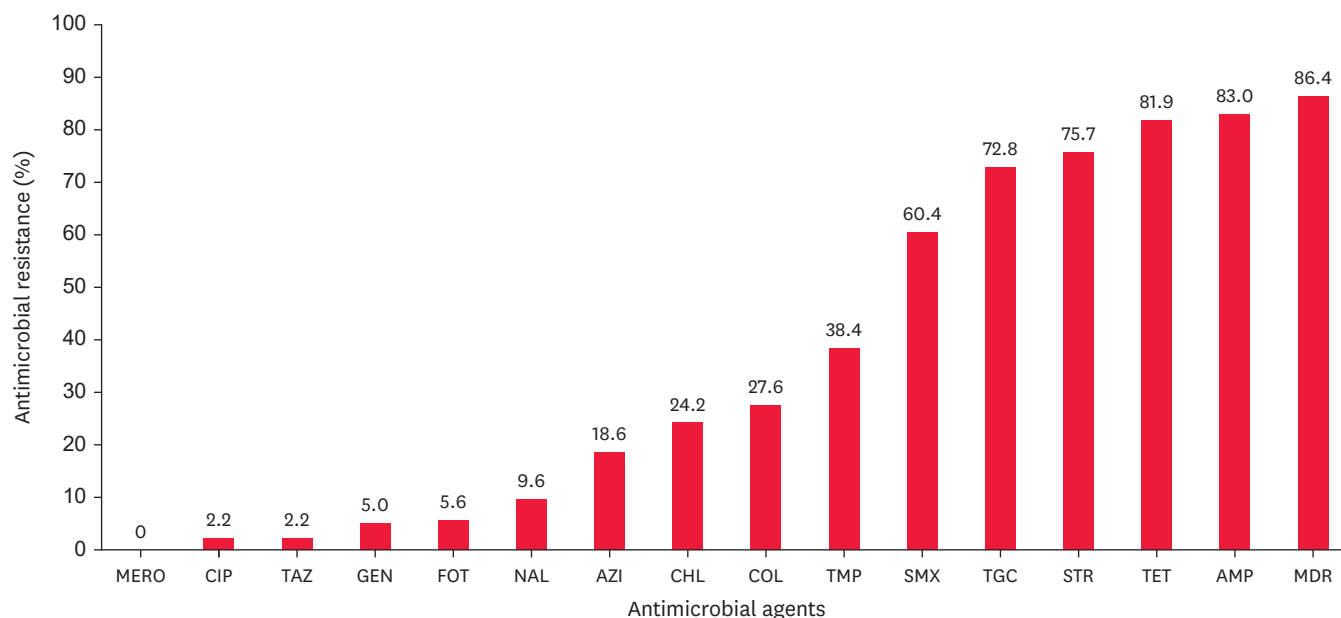
Fig. 1. Distribution of AMR among *E. coli* isolates in meat ducks (n = 177).
AMR, antimicrobial resistance; AMP, ampicillin; AZI, azithromycin; FOT, cefotaxime; TAZ, ceftazidime; CHL, chloramphenicol; CIP, ciprofloxacin; COL, colistin; GEN, gentamicin; MERO, meropenem; NAL, Nalidixic acid; STR, Streptomycin; SMX, sulfamethoxazole; TET, tetracycline; TGC, Tigecycline; TMP, trimethoprim; MDR, multidrug resistance.

Transfer of R plasmids and conjugation efficiency

Thirteen *E. coli* isolates transferred AMR conjugally in a single antibiotic selective pressure, including TET (n = 4/13), AMP (n = 3/13), and CHL (n = 3/13), of which the conjugation rates vary from 4.76×10^{-8} to 9.5×10^{-7} (**Table 3**). The three isolates transferred the plasmid horizontally in either AMP or TET selective pressure. None of the transconjugants were obtained when COL was used as a selective pressure. One of these three isolates (i.e., C249) transferred $bla_{\text{CTX-M1}}$ to corresponding transconjugants. All transconjugants exhibited

Table 3. Resistance phenotypes and plasmid replicon types of donors (n = 13) and their corresponding transconjugants (n = 16)

Donors				Transconjugants				Conjugation rate
ID	Resistance pattern	Inc group	FAB formula	Selective pressure	ID	Resistance pattern	Inc group	
A144	AMP-STR-TET-TGC	FrepB, FIC	F29:A-:B23	Ampicillin	A144Z1	AMP-TET-TGC	FrepB	4.76×10^{-8}
A183	AMP-STR-TET-TGC	FrepB	F29:A-:B-	Ampicillin	A183Z1	AMP	FrepB	9.5×10^{-7}
B206	AMP-STR-SMX-TET-TMP	-	-	Tetracycline	B206Z1	AMP-STR-SMX-TET-TGC-TMP	-	9.5×10^{-7}
B136	AMP-COL-STR-SMX-TET-TGC-TMP	-	-	Tetracycline	B136Z1	AMP-STR-SMX-TET-TMP	-	9.5×10^{-7}
B170	AMP-CHL-COL-STR-SMX-TET-TMP	-	-	Chloramphenicol	B170Z1	CHL	-	1.11×10^{-8}
B173	AMP-CHL-COL-STR-SMX-TET-TGC-TMP	-	-	Chloramphenicol	B173Z1	CHL-COL	-	2.7×10^{-7}
C248	AMP-CHL-COL-NAL-STR-SMX-TET-TGC-TMP	FIB, FIC, FrepB	F18:A-:B-	Chloramphenicol	C248Z1	CHL	FrepB	6.3×10^{-7}
C250	AMP-FOT-TAZ-CHL-COL-GEN-STR-SMX-TET-TGC	FrepB	F47:A-:B-	Tetracycline	C250Z1	AMP-AZI-COL-TET	-	2.1×10^{-7}
C200	AMP-STR-SMX-TET-TGC-TMP	-	-	Ampicillin	C200Z1	AMP-STR-SMX-TET-TGC-TMP	-	2.1×10^{-7}
A175	AMP-STR-TET	I1	-	Tetracycline	A175Z1	TET	-	9.5×10^{-7}
A198	AMP-FOT-TET-TGC	-	-	Ampicillin	A198Z1	AMP-FOT-TET	-	6×10^{-7}
				Tetracycline	A198Z2	AMP-FOT-TET	-	6×10^{-7}
C249	AMP-FOT-TAZ-CHL-COL-GEN-STR-SMX-TET-TGC	FrepB	F47:A-:B-	Ampicillin	C249Z1	AMP-FOT-TAZ-CHL-GEN-STR-TET-TGC	FrepB	2.1×10^{-7}
				Tetracycline	C249Z2	AMP-FOT-TAZ-CHL-GEN-STR-TET	FrepB	4.23×10^{-7}
C253	AMP-STR-TET-TGC	-	F4:A-:B-	Ampicillin	C253Z1	AMP-STR-TET-TGC	-	9.5×10^{-7}
				Tetracycline	C253Z2	AMP-STR-SMX-TET-TGC	-	1.9×10^{-8}

Inc, incompatibility; AMP, ampicillin; STR, Streptomycin; TET, tetracycline; TGC, Tigecycline; SMX, sulfamethoxazole; TMP, trimethoprim; COL, colistin; CHL, chloramphenicol; NAL, Nalidixic acid; FOT, cefotaxime; TAZ, ceftazidime; GEN, gentamicin; AZI, azithromycin.

resistance to other antibiotics in addition to the antibiotic selection pressures, and most were multidrug resistant. Some transconjugants selected by AMP and TET (i.e., C249Z1 and C249Z2, respectively) were resistant to CHL. At the same time, CHL and TET were used to select the transconjugants resistant to COL (i.e., B173Z1 and C250Z1, respectively).

Plasmid replicon types (n = 29) and replicon sequence types of IncF plasmids (n = 6)

Five replicon types were found among the *E. coli* donors (n = 13) and their respective transconjugants (n = 16). The most common replicon identified in *E. coli* donors was IncFrepB (n = 5/13), followed by IncFIC (n = 2/13). The other replicons identified were IncI$_1$ (n = 1/13), IncY (n = 1/13) and IncFIB (n = 1/13). The most common replicon identified among the transconjugants was IncFrepB (n = 5/16). Although IncFrepB was found in the donors and transconjugants, some donors and transconjugants did not carry Inc plasmids tested in this study. The six *E. coli* isolates with IncF replicon possessed different IncF replicon sequence types according to pMLST analysis. Five FAB formulas were identified including C249 and C250, F47:A-:B-; A144, F29:A-:B23; A183, F29:A-:B-; C248, F18:A-:B- and C253, F4:A-:B- (**Table 3**).

Association among AMR phenotype in *E. coli* isolates (n = 177)

Different associations among the AMR phenotypes in the *E. coli* isolates (n = 177) were observed (**Table 4**). Overall, the resistance phenotypes showed more positive than negative relationships with each other. The strongest positive association was observed between AMP and TET resistance (OR, 50.3; confidence interval [CI], 17–148), followed by CHL and SMX resistance (OR, 44.5; CI, 5.9–333). Chloramphenicol resistance was positively associated with all antibiotics tested except for TGC. A strong positive association (OR > 10) was observed between CHL resistance and resistance to COL, GEN, SMX, and TET. No positive association was observed between MDR and the AMR phenotype, but some showed a significant association ($p < 0.05$).

DISCUSSION

The published data on livestock was also used to compare the findings in this study because there is currently limited information on AMR in bacteria originating from meat ducks. One of the key findings was the high percentage of MDR commensal *E. coli* (86.4%) from meat ducks raised in the open house system. The *E. coli* isolates in this collection exhibited a greater percentage of MDR than those from ducks in Tanzania [33] and Korea [34] but comparable to that in pigs, pig carcasses, and humans in Thailand [23]. As *duck manure* is *usually discharged directly into the rearing area*, the significant role of meat ducks in the environmental dissemination of AMR bacteria and their resistance determinants was underlined.

The *E. coli* isolates in this study exhibited the highest resistance rates to AMP (83.0%) and TET (81.9%), surpassing those observed in a previous study [34]. Prolonged use of antibiotics in food animal production, including duck production, was likely the cause of such a high resistance rate. The high AMP resistance corresponded well to the fact that amoxicillin is the most used antibiotic in duck farming. Ampicillin has been suggested to be included in the antimicrobial panel for AMR monitoring [12]. This was attributed to cross-resistance between AMP and amoxicillin, suggesting that AMP-resistant bacteria are mostly resistant

Table 4. Association between AMR phenotypes in *E. coli* isolates (n = 177)

	No.[a]	AMP	AZI	FOT	CHL	COL	GEN	NAL	STR	SMX	TET	TGC	TMP
AMP	147	NS	8.0 (1.0–61.5)	-[b]	1.2 (1.1–1.4)	14.6 (1.8–106)	-	-	-	-	50.3 (17–148)	-	11.4 (2.6–49.6)
AZI	33	8.0 (1.0–61.5)	NS	-	3.4 (1.5–7.6)	-	3.8 (0.9–15.1)	-	-	2.3 (0.9–5.5)	1.2 (1.1–1.4)	3.1 (1–9.6)	-
FOT	10	-	-	NS	5.2 (1.4–19.6)	4.3 (1.1–1)	21.6 (4.5–101)	-	-	-	-	-	-
TAZ	4	-	-	28.8 (13.1–63.2)	4.4 (3.3–5.8)	3.8 (2.9–2.9)	34.6 (14.5–82)	-	-	-	-	-	-
CHL	43	1.2 (1.1–1.4)	3.4 (1.5–7.6)	5.2 (1.4–19.6)	NS	24.3 (10–58)	30.4 (3.6–251)	7.3 (2–21)	3 (1.1–8.2)	44.5 (5.9–333)	12.6 (1.6–95)	-	7.9 (3.6–17.3)
CIP	4	-	-	-	4.4 (3.3–5.8)	8.2 (0.8–82.6)	-	-	-	-	-	-	2.7 (2.2–3.28)
COL	49	14.6 (1.8–106)	-	4.3 (1.1–16.0)	24.3 (10–58)	NS	10.5 (2–52.0)	4.3 (1.5–12)	3.7 (1–10)	4.8 (2–11)	7.1 (1.6–31.3)	2.8 (1.1–6.8)	3.3 (1.6–6.5)
GEN	9	-	3.8 (0.9–15.1)	21.6 (4.5–101)	30.4 (3.6–251)	10.5 (2.5–51)	NS	-	-	1.7 (1.5–1.9)	-	-	-
NAL	17	-	-	-	7.3 (2.5–21.3)	4.3 (1.5–12)	-	NS	1.3 (1.2–1.5)	5.5 (1.2–25)	-	6.6 (0.8–51.6)	6.2 (1.9–19)
STR	134	-	-	-	3 (1.1–8.2)	3.7 (1.36–10)	-	1.3 (1.2–1.5)	NS	2.4 (1.2–4.8)	-	-	3.5 (1.5–8.2)
SMX	107	-	2.3 (0.9–5.5)	-	44.5 (5.9–333)	4.8 (2–11)	1.7 (1.5–1.9)	5.5 (1.2–25)	2.4 (1.2–4.8)	NS	-	-	5.3 (2.5–11)
TET	145	50.3 (17–148)	1.2 (1.1–1.4)	-	12.6 (1.6–95)	7.1 (1.6–31.3)	-	-	-	-	NS	-	3.2 (1.2–8.3)
TGC	129	-	3.1 (1–9.6)	-	-	2.8 (1.1–6.8)	-	6.6 (0.8–51)	-	-	-	NS	-
TMP	68	11.4 (2.6–49.6)	-	-	7.9 (3.6–17.3)	3.3 (1.6–6.5)	-	6.2 (1.9–19)	3.5 (1.5–8.2)	5.3 (2.5–11)	3.2 (1.2–8.3)	-	NS

Data shown are odds ratio for significant associations between AMR phenotypes (95% confidence interval in parenthesis); OR>1 and <1 shows positive and negative associations respectively.
AMR, antimicrobial resistance; AMP, ampicillin; AZI, azithromycin; FOT, cefotaxime; CHL, chloramphenicol; COL, colistin; GEN, gentamicin; NAL, nalidixic acid; STR, streptomycin; SMX, sulfamethoxazole; TET, tetracycline; TGC, tigecycline; TMP, trimethoprim; NS, no statistics determined.
[a]Number of isolates resistant to corresponding antimicrobial agents.
[b]No statistically significant ($p \geq 0.05$).

to amoxicillin. The resistance to TET was more common than in Tanzania [33], but lower than that of the pathogenic *E. coli* from ducks in China [35]. The differences could be brought about by differences in geographical location, availability of antibiotics, antimicrobial usage form, and antibiotics administration. Future antimicrobial use (AMU) situation analysis is suggested to comprehend the AMR dynamics. Compared to livestock, the high AMP and TET resistance rates agree with previous studies conducted in commensal *E. coli* from different sources, e.g., pigs in Vietnam [36], hens in Thailand [37], healthy swine in Thailand [38], and chickens in China [39]. In addition, a low resistance rate to CIP (2.2%) was observed, which is in line with *E. coli* from pigs in Vietnam [36] and Thailand [21].

A particular concern has been raised about the emergence and spread of resistant bacteria of food animal origin to last-resort antibiotics (e.g., COL, MERO, and third-generation cephalosporins) that may enter the food chain and the environment. In this study, 28% of *E. coli* were resistant to COL, inconsistent with a study on ducks [34] but similar to previous studies on livestock and poultry. Colistin is one of the highest priorities and critically important antibiotics, but it has been used extensively in pig farming. No participating farms in this study declared the use of COL. The conjugation experiment suggested that COL resistance was transferred when CHL (in B173Z1) and TET (in C250Z1) were used as the selective pressure. This observation points to co-selection mediated by other antibiotics as a mechanism contributing to the presence of COL-resistant *E coli*. On the other hand, other

possible reasons exist, e.g., environmental contamination and transmission from workers and other animals. Therefore, further studies are needed to understand the dynamics of COL resistance in the interconnected ecosystems of humans, animals, and environmental health.

In contrast, the FOT (5.6%) and TAZ (2.2%) resistance rates were also still at limited levels, and no MERO resistance was found, in agreement with a previous study [34]. Very limited resistance to these last resorts could be attributed to their limited use in meat duck production. Despite the low resistance rates, commensal *E. coil* resistant to COL and third-generation cephalosporins raised alarm bells about the prevalence of the relevant genes that are mostly plasmid-encoded.

ESBL-producing *E. coli* was found (5.0%, n = 9/177) at a lower level than previous studies conducted in backyard ducks (36.6%) and chickens (24.9%) in Thailand [40] and backyard ducks (> 50%) in China [41]. Ducks generally discharge their feces directly into water reservoirs, facilitating the rapid spread of ESBL-producing *E. coli* among duck populations and the environment. Despite the small percentage of ESBL-producing *E. coli* observed, all were MDR. This is consistent with ESBL producers frequently displaying MDR [42].

Conjugal transfer was observed under the selection pressure of AMP, TET, and CHL. In contrast, no transconjugants were obtained under COL selective pressure, consistent with a previous study in food animals in China [43]. This may be due to the lack of mobile genetic elements carrying COL resistance genes, plasmid incompatibility, low transfer frequency, and experimental conditions [44]. The *in vitro* conjugation of plasmids may not fully mirror the phenomena in vivo because of the absence of some factors that lower the transfer efficacy under *in vitro*.

Despite the ban from being used in food animals since 1994, CHL-resistant *E. coli* isolates were isolated in this study, which agrees with previous livestock studies [45]. It was suggested to be the consequence of co-selection and cross-resistance induced by other antibiotics [46]. This agrees with the results in this study, where AMP and TET were co-selected for CHL-resistant transconjugants (i.e., C249Z1 and C249Z2).

The correlation between the AMR phenotypes varied. The strongest association was between AMP and TET resistance (OR, 50.3), consistent with the high resistance rates to these two antibiotics, suggesting that the corresponding resistance genes co-localized on the same genetic elements. Chloramphenicol resistance was positively associated with almost all antibiotics tested, with a strong correlation (OR > 10) with resistance to the antibiotics commonly used in livestock and poultry (e.g., GEN [OR > 30.4] and SMX [OR > 44.5]). The results indicate the co-localization of genes encoding CHL resistance and resistance to other antibiotics on the same genetic elements. Hence, the genes encoding resistance to CHL and others co-localize on the same genetic elements. Therefore, initiatives to regulate antimicrobials should be carried out from a whole-system perspective.

IncF is a predominant Inc group in Enterobacteriaceae [32], which agrees with the current study. The IncFrepB plasmid was found in most MDR *E. coli* from meat ducks in this collection, which is in agreement with previous studies on livestock [22]. Interestingly, some donors and their corresponding transconjugants did not carry the same Inc plasmids despite the transfer of resistance phenotype (i.e., C250/C250Z1 and C175/A175Z1). The latter may be because the bacterial strains carry plasmids in Inc groups that were not detected by the PBRT

scheme used in this study, suggesting that the detection scheme of Inc plasmids should be expanded to cover many other different Inc groups.

In conclusion, the commensal *E. coli* from meat ducks in this study exhibited high rates of AMR, with the majority demonstrating MDR. The results highlight the significant role of meat ducks as reservoirs for *E. coli* with a range of plasmids and AMR determinants present in duck manure released directly into the environment. They may transfer to humans through the food chain. The authors fervently advocate for including meat duck-associated bacteria in AMR monitoring and surveillance programs as an element of the One Health concept. Nationwide monitoring and surveillance to collect and track AMU data in ducks raised for food should be established and conducted routinely. The strategic actions to reduce the emergence and prevent the spread of AMR in livestock should also be expanded to meat duck production.

ACKNOWLEDGMENTS

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