Veterinary Integrative Sciences 2024; 22(3): 769 - xxx DOI; 10.12982/VIS.2024.051

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

Prevalence and antimicrobial resistance profile of *Escherichia* coli O157:H7 isolated from chickens and chicken meats from local poultry slaughterhouses in Nakhon Ratchasima Province, Thailand

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Abstract

This study sought to investigate the antimicrobial resistance profiles of *Escherichia coli* O157:H7 (EOH) isolated from chickens and chicken products at local poultry slaughterhouses in Nakhon Ratchasima Province, Thailand. EOH was isolated from cloacal swabs and chicken breast meat samples between January 2021 and June 2023. EOH were identified using a culture-based method and were confirmed on sorbitol MacConkey agar. Positive EOH isolates were tested via antimicrobial susceptibility testing and antimicrobial resistance profiles using the disk diffusion method and polymerase chain reaction. This study showed the prevalence of EOH isolated from chicken breast meat (11.47%) was higher than that of cloacal swab (9.32%). Most EOH isolates from cloacal swabs were resistant to oxytetracycline (65.38%), streptomycin (61.54%), amoxicillin (61.54%), and ampicillin (65.38%, and most EOH isolates from breast meat were resistant to oxytetracycline (78.12%), streptomycin (25%), amoxicillin (75%), and ampicillin (78.12%). In addition, 32 (55.17%) EOH isolates were multidrug resistant harbored the ESBL-TEM gene (65.38%), bla_{TEM} (34.61%), *int-1* (3.85%), or *mcr-1* (0%). This is the first report of EOH isolated from chickens and chicken meats from domestic poultry slaughterhouses in Nakhon Ratchasima Province. Therefore, EOH is a public health concern in local poultry slaughterhouse processes in this area.

Keywords: Antimicrobial resistance, Chickens, *Escherichia coli* O157:H7, Multidrug resistance

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Article history; *received manuscript: 26 June 2023,*
revised manuscript: 6 August 2023,
accepted manuscript: 26 December 2023,
published online: 3 January 2024

Academic editor: *Korakot Nganvongpanit*

INTRODUCTION

Escherichia coli O157:H7 (EOH) and commensal *E. coli* are important food-borne pathogenic bacteria of public health concern (Fuh et al., 2018). EOH has been found in chicken intestines, chicken meats, and chicken meat products and can survive at freezing temperature (−20°C) and in acidic food (Miri et al., 2022). Chickens are easy and cheap sources of animal protein (Sarangi et al., 2016; Wardhana et al., 2021), and chicken meat, and intestines are a rich source of *E. coli* (Ranasinghe et al., 2022). Furthermore, chicken meat is a common source of EOH that can produce further contamination during chicken meat processing, including during the slaughtering process (Fuh et al., 2018; Fahim et al., 2020). Antimicrobial resistance (AMR) and multidrug resistance (MDR) in *E. coli* is a global public health concern (Asai et al., 2022). The spread of MDR is becoming a key cause of mortality involving longer hospital stays and expensive treatments (Matthew et al., 2008; Jyoti et al., 2014). Bacteria exhibiting MDR are resistant to at least three antimicrobial classes, leading to more outbreaks of bacteria with AMR worldwide. Extended spectrum beta-lactamase (ESBL) gene in gram-negative bacteria causes resistance to the broad-spectrum beta-lactam antimicrobials (Adamu et al., 2014). Beta-lactamase (*bla*) gene in gram-negative bacteria causes resistance to the narrow spectrum beta-lactam antimicrobials (Kiratisin et al., 2012). The class 1 integron (*int-1*) gene is part of integron structure (Kiratisin et al., 2012) and *mcr-1* is a colistin drug resistance gene (John, 2018), which can be transmitted horizontally between animals, humans, and the environment via bacteria (Gharaibeh et al., 2019). Most genes and genetic elements encoding AMR found in pathogenic *Enterobacteriaceae* are extended-spectrum beta-lactamase (ESBL), beta-lactamase (*bla*), class 1 integrase (*int-1*), and colistin (CT) resistance (*mcr-1*) genes. Although ESBL-encoding genes, particularly TEM, have been frequently detected in chickens and chicken meat worldwide (Antunes et al., 2016), a class 1 integron has been identified as the most common resistance integron (Guran et al., 2020). This integron comprises gene cassettes that enable the distribution of AMR in most gram-negative enteric bacteria and that consequently exert a considerable influence on the antimicrobial nature of MDR in many bacterial species (Guran et al., 2020). Additionally, the horizontal transmission or conjugation of CT-resistant Enterobacteriaceae, and especially *E. coli* carrying *mcr-1*, among animals used for livestock, humans, and environments has been reported to occur globally (Eiamphungporn et al., 2018; Sakdinun et al., 2018). Among 77chicken producing provinces, Nakhon Ratchasima Province is the major area for broiler production in Thailand. According to Department of Livestock Development (2019), Nakhon Ratchasima is one of the three main provinces producing the highest number of broilers in Thailand, of which Lopburi, Kanchanaburi, and Nakhon Ratchasima, produces 18.58%, 9.85%, and 9.74%, respectively of total broiler production in Thailand (Department of Livestock Development, 2019). Therefore, this study aimed to investigate the prevalence and AMR profile of EOH isolated from chickens and their meat products at local slaughterhouses in Nakhon Ratchasima Province, Thailand.

MATERIALS AND METHODS

Ethical approval

The use of animals in the current study was approved with permission and following the guidelines of the Institutional Animal Care and Use Committee of Khon Kaen University (KKU) as described in permission record no. IACUC-KKU-79/64 and reference no.660201.2.11/374 (86).

Sample size calculation in the methodology

The formula had shown sample size calculation for prevalence value of research.

Sukon (2013) and Naing (2006) reported that the following simple formula was used:

$$N = \frac{Z^2P(1 - P)}{L^2} \quad \text{or} \quad \frac{4P(100 - P)}{25}$$

N = Samples size
Z = Z statistic = 1.96 (For the level of confidence of 95%), $Z^2 \approx 4$
P = expected prevalence or proportion or prevalence of bacteria (%)
L = accepted error = 5 (confidence interval 95.0% and accepted error 5%)

Sample size determination in Thailand

P = 40 (Elsharawy et al., 2022) from pathogenic bacteria (*Escherichia coli* O157:H7)

$$N = \frac{4 \text{ X } 40 \text{ X } (100 - 40)}{25}$$

N = 384, which represents the minimum sample size for this research.

Therefore, chicken fresh feces and breast meats were collected from consented local poultry slaughterhouses in Nakhon Ratchasima Province, Thailand and were at least 384 samples.

Study period and location

The study was conducted from January 2021 to June 2023. Samples were collected from chicken in consented local poultry slaughterhouses at Nakhon Ratchasima Province. Nine cloacal swabs and chicken breast meat samples were randomly collected from each of 31 domestic poultry slaughterhouses from 50 chicken farms in 12 districts in Nakhon Ratchasima Province, Thailand, using a cross-sectional design. All farms and slaughterhouses have been certified to raise and slaughter chickens in Thailand by the Thai government. A total of 558 samples were collected (279 from cloacal swabs and chicken breast meat) from various chicken types, comprising broilers (432 samples), indigenous chickens (90 samples), and spent laying hens (36 samples). Samples (from pre- and post-slaughtered chicken) were kept at 4°C and transferred to a microbiology laboratory at the Department of Veterinary Public Health, Faculty of Veterinary Medicine, Khon Kaen University, Thailand, within 24 h. Mind mapping of the study design is shown in Figure 1. Additionally, total 558 samples were

collected from 31 local poultry slaughterhouses in Nakhon Ratchasima Province. Map of sample collection centers was prepared using the ArcGIS Program, Figure 2 shows geographical location of sampling point locations of local poultry slaughterhouses in Nakhon Ratchasima Province, Thailand (mark S is the number of local poultry slaughterhouses in the district area of Nakhon Ratchasima Province).

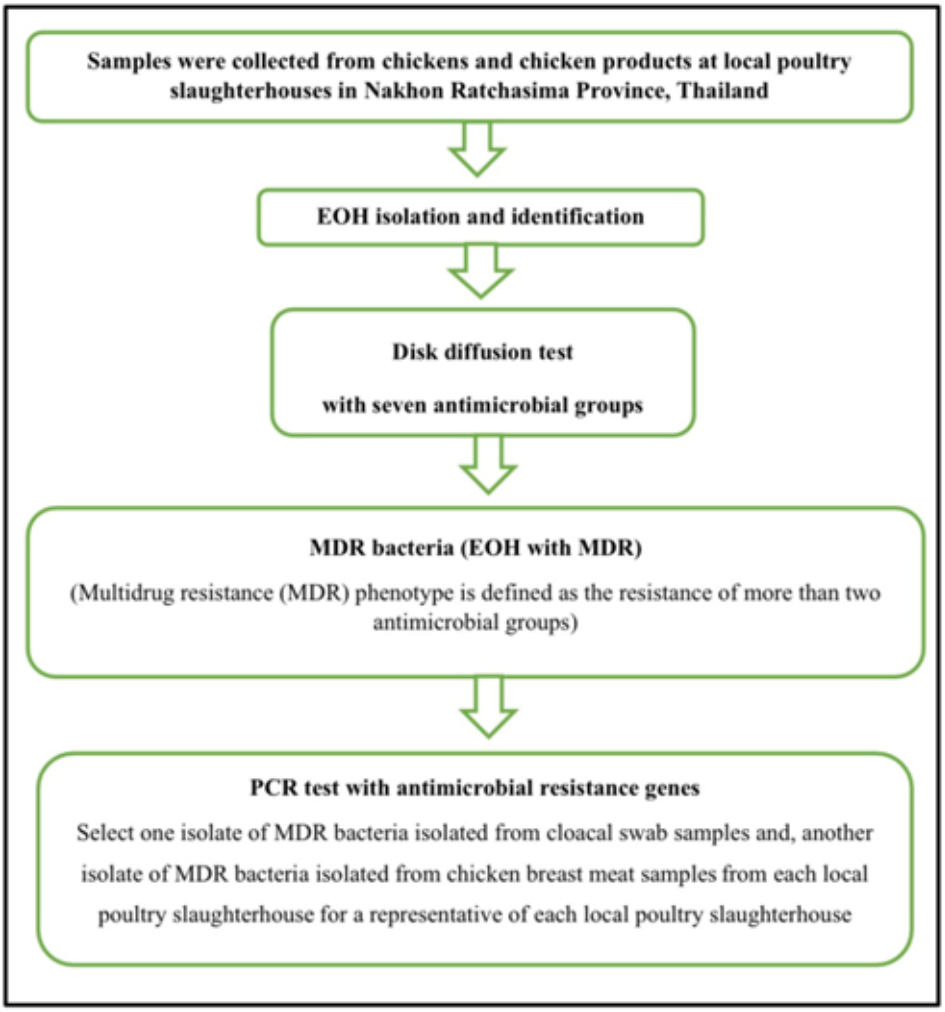

Figure 1 Mind mapping of study design

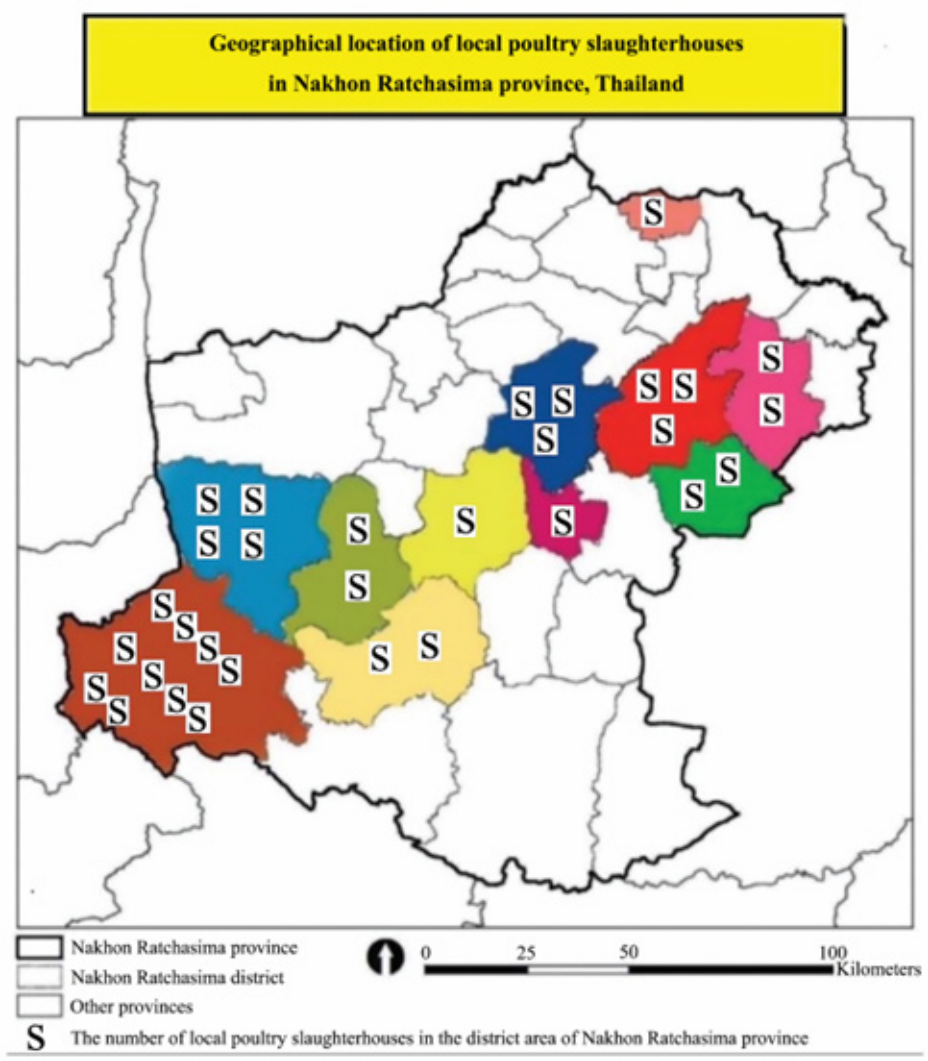

Figure 2 Map showing samples collection area in Nakhon Ratchasima Province, Thailand

EOH isolation

Chicken breast meat (25 g) was mixed in 225 mL of buffered peptone water (BPW, Oxoid Ltd., Basingstoke, U.K.) for 3 min at temperature room using a stomacher while each cloacal swab was mixed in 10 mL of BPW and then incubated at 37°C for 16–24 h. Subsequently, the BPW was steaked on MacConkey agar (Oxoid Ltd., Basingstoke, U.K.), incubated at 37°C for 24 h (Bhoomika et al., 2016). One pink colony from the MacConkey agar was streaked on xylose lysine deoxycholate agar (XLD, Oxoid Ltd.) for double-confirm *E.coli* and *E.coli* O157:H7 by using selective media and then incubated at 37°C for 18–24 h. Subsequently, one yellow colony from XLD agar was streaked on sorbitol MacConkey agar (Oxoid Ltd.) and incubated at 37°C for 18–24 h, which *E. coli* O157:H7 is identified by culture on selective indicator media such as Sorbitol MacConkey Agar or the same agar containing cefixime and tellurite (Adamu et al., 2014). A yellow or straw colony from sorbitol MacConkey agar was then assessed by the methods of Adamu et al. (2014). Additionally, EOH colonies were identified morphologically, microscopically, and by biochemical tests and were then assessed by the methods of Elsharawy et al. (2022a), Elsharawy et al. (2022b), and Shecho et al. (2017). Furthermore, EOH was identified by culture on sorbitol MacConkey agar, which is a selective,

and differential medium for the detection of *E. coli* O157, where the positive control (EOH nontoxigenic NCTC 12900) produced 1–2-mm sized straw colonies as shown in Figure 3, whereas the negative control (*E. coli* ATCC 25922) produced 1–2-mm sized pink colonies as shown in Figure 3 (Adamu et al., 2014). Next, one straw colony from sorbitol MacConkey agar was then streaked on eosin methylene blue agar (EMB, Oxoid Ltd.) and incubated at 37°C for 18–24 h. Positive colonies from EMB agar (dark-centered and flat colonies with metallic sheen) were assessed using the method of Bhoomika et al. (2016), Shecho et al. (2017), Elsharawy et al. (2022a) and Elsharawy et al. (2022b). These colonies were then streaked on triple sugar iron agar (Oxoid Ltd.) and motility indole lysine medium (Oxoid Ltd.) and incubated at 37°C for 18–24 h. Subsequently, positive colonies was placed in cryovial sterile tubes (600 µL buffered peptone water and 200 µL 20% glycerol) and stored at −80°C.

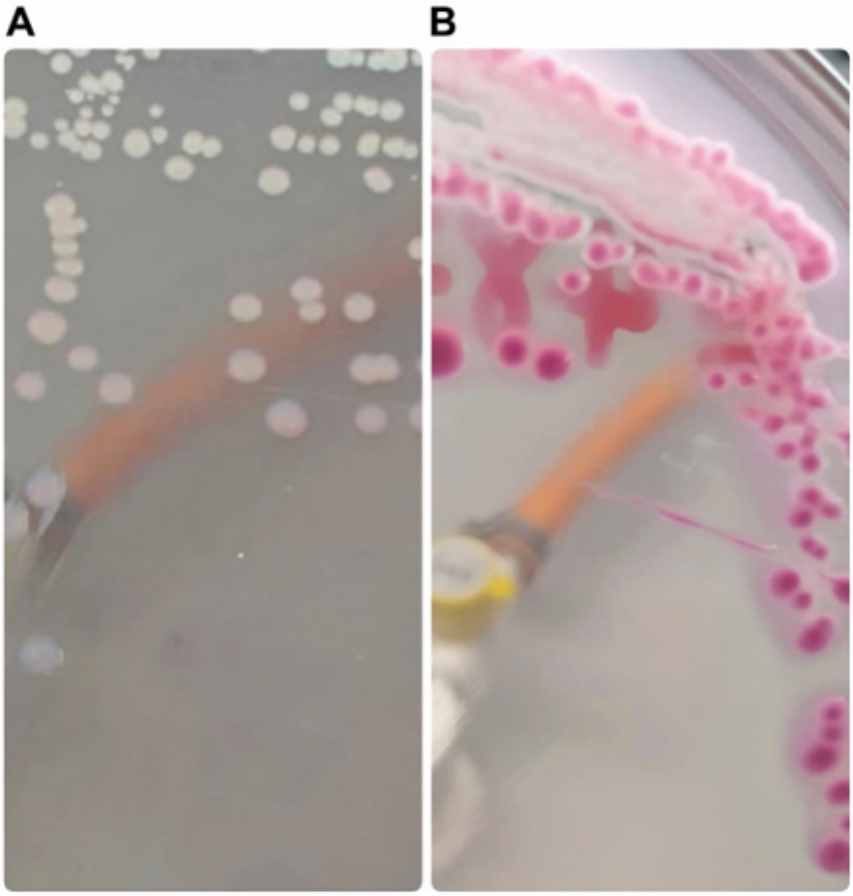

Figure 3 EOH were culture on sorbitol MacConkey agar (A), and E. coli ATCC 25922 were culture on sorbitol MacConkey agar (B).

Antimicrobial susceptibility test

Antimicrobial susceptibility tests were performed using a modified version of Kirby–Bauer disk diffusion method (Bauer et al., 1966). Mueller Hinton agar (MHA, Oxoid Ltd.) were used in disk diffusion test for EOH and was tested with seven antibiotic groups, including beta-lactams (cephalexin, CL; cefepime, FEP; cefotaxime, CTX; cefoxitin, FOX, amoxicillin, AML; ampicillin, AMP), tetracyclines (oxytetracycline, OT; doxycycline, DO), fluoroquinolones (enrofloxacin), antimicrobial peptides (CT), chloramphenicol, aminoglycosides antimicrobial group (neomycin, N; streptomycin, S), and sulphonamides (sulphamethoxazole/trimethoprim, SXT) (14 antibiotic disks) (Oxoid Ltd).

Bacterial inoculum preparation for disk diffusion test

Direct colony suspension was employed by suspending EOH colonies in 2 mL of 0.85% (w/v) normal saline. Then, the inoculum was adjusted to a 0.5 McFarland standard (1.5×10^8 CFU/mL). Furthermore, broth cultures were adjusted to a 0.5 McFarland standard turbidity in sterile normal saline. A sterile cotton swab was then dipped in the turbidity adjusted culture and was then swabbed in three directions on a MHA plate. Inoculated plates were incubated at 37°C for 24 h.

Interpretation of disk diffusion test

The diameter of inhibition zones was measured with vernier calipers and a calibrated ruler. Interpretations were made referring to the zone diameter interpretive standards from Clinical and Laboratory Standard Institute (CLSI, 2022). Additionally, multidrug resistance (MDR) is defined as the resistance to more than two antimicrobial groups or antimicrobial resistance in species of microorganism to at least three antimicrobial groups (Matthew et al., 2008; Kiratisin et al., 2012; Peter et al., 2021). Hence, MDR isolates of this study are defined as bacteria have Multidrug resistance (MDR) status.

PCR amplification from EOH colonies

One EOH colony exhibiting MDR that was isolated from cloacal swab samples and from chicken breast meat samples, representing each of the local poultry slaughterhouses, was used for PCR testing. DNA was extracted from the colonies by boiling in 100 µL of deionized water for 30 s at 100°C. The specific primer sequences and PCR conditions (Hot start Tag DNA polymerase 500 u Brand (Biotech rabbit, Germany), antimicrobial resistance gene-specific primers, template DNA (bacteria isolated), VC100bp Plus DNA ladder (ready-to-use), 50 µg (Vivantis, Malaysia), 6X DNA Loading dye (Thermo scientific, EU), 10X TAE buffer (Axil Scientific, Singapore), PCR machine or Thermal cycler, Ethidium bromide solution 1.0% (AppliChem, Germany), Agarose gel 1.5% (Genepure LE, Spain), Electrophoresis machine, and UV-transilluminator machine) used for amplification of antimicrobial resistance genes are shown in Table 1.

Table 1 Antimicrobial resistance genes specific primers used in this study

Gene	Sequence (5′–3′) (F = forward, R = reverse)	Annealing Temperature (°C)	Amplicon size (bp)	References
ESBL-TEM F	TTTCGTGTCGCCCTTATTCC	50	404	(Lertworapreecha et al., 2016)
ESBL-TEM R	ATCGTTGTCAGAAGTAAGTTGG	50	404	(Lertworapreecha et al., 2016)
bla_{TEM} F	CATTTCCGTGTCGCCCTTAT	55	793	(Lertworapreecha et al., 2016)
bla_{TEM} R	TCCATAGTTGCCTGACTCCC	55	793	(Lertworapreecha et al., 2016)
int-1 F	GGGTCAAGGATCTGGATTTCG	62	483	(Lapierre et al., 2020)
int-1 R	ACATGGGTGTAAATCATCGTC	62	483	(Lapierre et al., 2020)
mcr-1 F	AGTCCGTTTGTTCTTGTGGC	58	320	(Lapierre et al., 2020)
mcr-1 R	AGATCCTTGGTCTCGGCTTG	58	320	(Lapierre et al., 2020)

Statistical analysis

The MDR pattern is described as resistance to antibiotics in a minimum of three antimicrobial classes. First, positive *Escherichia coli* O157:H7 isolates from chicken cloacal swabs, chicken breast meat, and phenotypic/genotypic AMR profiles were reported in percentages. Then, the Chi-square test was used to test the relationship between the diverse prevalence of chicken cloacal swabs and chicken breast meat samples in *Escherichia coli* O157:H7 isolates and their phenotypic/genotypic AMR profiles. Finally, statistical analyses were performed using SPSS (v. 16.0; SPSS Inc., Chicago, IL, USA), and we considered the significant difference at $p < 0.05$.

RESULTS

Prevalence of EOH and EOH with MDR isolated from cloacal swabs and chicken breast meat

The prevalence of *E. coli* isolated from cloacal swab samples (84.23%) was significantly more than that of *E. coli* isolated from chicken breast meat samples (63.80%; $p < 0.05\%$) from domestic poultry slaughterhouses in Nakhon Ratchasima Province, Thailand. However, the prevalence of EOH isolated from chicken breast meat samples (11.47%) was not significantly different from that of EOH isolated from cloacal swab samples (9.32%). Overall, the prevalence of *E. coli* (74.01%) among all samples was more than that of EOH (10.39%) (Table 2).

Table 2 Prevalence of EOH and *E. coli* isolated from cloacal swab and chicken breast meat

Bacterial strain	Samples	
	Cloacal swab	Breast meat
EOH	26/279 (9.32%)	32/279 (11.47%)
Total	58/558 (10.39%)	
E. coli	235/279 (84.23%)	178/279 (63.80%)
Total	413/558 (74.01%)	

Phenotype of antimicrobial resistance of EOH isolated from cloacal swabs and chicken breast meat samples

The present study shows that the total of EOH with MDR is 5.73%. Furthermore, EOH isolates exhibiting MDR were identified from chicken breast meat than from cloacal swabs. Similarly, EOH isolated from cloacal swab exhibited 11 MDR profiles while EOH isolated from chicken breast meat exhibited 12 MDR profiles (Table 3). Additionally, the positive rate of EOH isolates with MDR from cloacal swab samples was less than that of EOH isolated from chicken breast meat samples. Similarly, the EOH isolated from cloacal swab and chicken breast meat samples had the largest number of isolates with AMR (10 and 9, respectively)

Most EOH isolates from cloacal swab samples exhibited an MDR profile of AML-AMP-S-OT resistance while that of breast meat samples was CL-FOX-AML-AMP-ENR-DO-OT resistance. Most EOH isolates from cloacal swab samples were resistant to OT, S, AML, and AMP (65.38%, 61.54%, 61.54%, and 65.38%, respectively) (Table 3). Furthermore, most EOH isolates from chicken breast meats were resistant to OT, S, AML, and AMP (78.12%, 25%, 75%, and 78.12%, respectively) Moreover, more EOH isolates from cloacal swab samples were resistant to S than EOH isolates from breast meat samples ($p < 0.05$). The AMR and MDR profiles of EOH isolates are shown in Table 3, Table 4, and Table 5.

Table 3 Phenotypic antimicrobial-resistant EOH isolated from domestic poultry slaughterhouses in Nakhon Ratchasima Province

Sample	AMR[a] (no. of isolates) to antimicrobial agents (%)													
	AML	AMP	C	CL	CT	CTX	DO	ENR	FEP	FOX	N	OT	S	SXT
Cloacal swab	61.54 (16/26)	65.38 (17/26)	11.54 (3/26)	26.92 (7/26)	7.69 (2/26)	7.690 (2/26)	38.46 (10/26)	23.08 (6/26)	3.85 (1/26)	23.08 (6/26)	19.23 (5/26)	65.38 (17/26)	61.54 (16/26)	23.08 (6/26)
Breast meat	75 (24/32)	78.12 (25/32)	3.12 (1/32)	50 (16/32)	3.12 (1/32)	6.25 (2/32)	59.37 (19/32)	37.5 (12/32)	0 (0)	43.75 (14/32)	12.5 (4/32)	78.12 (25/32)	25 (8/32)	9.37 (3/32)
p-value	0.27	0.28	0.316	0.074	0.582	1.000	0.186	0.238	0.448	0.099	0.717	0.28	0.005*	0.274

Antimicrobial agents: AML, amoxicillin; AMP, ampicillin; C, chloramphenicol; CL, cephalexin; CT, colistin; CTX, cefotaxime; DO, doxycycline; ENR, enrofloxacin; FEP, cefepime; FOX, cefoxitin; N, neomycin; OT, oxytetracycline; S, streptomycin; SXT, sulphamethoxazole/trimethoprim.
[a]Antimicrobial resistance
*The AMR profile between cloacal swabs and breast meat samples was significantly different (p 0.05).

Table 4 Prevalence of MDR status of EOH isolated from cloacal swab and chicken breast meat samples from domestic poultry slaughterhouses in Nakhon Ratchasima Province

Type of sample	EOH isolates (%)	EOH isolates with MDR (%)	p-value
Cloacal swab samples	26/279 (9.32%)	15/279 (5.38%)	0.728
Breast meat samples	32/279 (11.47%)	17/279 (6.09%)	
Total	58/558 (10.39%)	32/558 (5.73%)	

Note: The data about the relationship between the type of sample and the MDR status of EOH were analyzed by using Chi-square tests of the SPSS Statistics program software for Windows, Version 16.0; the significance level was set at 95% confidence interval ($p < 0.05$).

Table 5 Chicken cloacal swabs (CS) and Breast meat (BM) AMR[1] EOH profiles from local slaughterhouses in Nakhon Ratchasima Province, Thailand

Sample	No. of AM[2]	No. of AM classes	Resistance phenotypic profiles	No. of isolates (%)	p value[3]
CSBM	4	3	AML-AMP-S-OT	3 (5.17)	0.648
	4	3	AML-AMP-S-OT	2 (3.45)	
CS	4	4	C-S-SXT-OT	1 (1.72) (ND[4] in BM)	0.448
CS	5	4	AML-AMP-S-SXT-OT	1 (1.72) (ND in BM)	0.448
CS	6	5	AML-AMP-C-S-SXT-OT	1 (1.72) (ND in BM)	0.448
CS	7	3	CL-FOX-AML-AMP-ENR-DO-OT	2 (3.45) (ND in BM)	0.197
CS	7	4	FOX-FEP-AMP-CT-ENR-DO-OT	1 (1.72) (ND in BM)	0.448
CS	7	4	CL-AML-AMP-ENR-SXT-DO-OT	1 (1.72) (ND in BM)	0.448
CS	7	5	AML-AMP-C-S-SXT-DO-OT-	1 (1.72) (ND in BM)	0.448
CS	7	4	AML-AMP-N-S-SXT-DO-OT	1 (1.72) (ND in BM)	0.448
CS	8	3	CL-FOX-AML-AMP-N-S-DO-OT	1 (1.72) (ND in BM)	0.448
CS	10	4	CL-FOX-CTX-AML-AMP-ENR-N-S-DO-OT	2 (3.45) (ND in BM)	0.197
BM	4	3	AMP-SXT-DO-OT	1 (1.72) (ND in CS)	1.000
BM	6	5	AML-AMP-ENR-S-SXT-OT	1 (1.72) (ND in CS)	1.000
BM	6	3	CL-FOX-AML-AMP-ENR-OT	1 (1.72) (ND in CS)	1.000
BM	6	4	AML-AMP-ENR-S-DO-OT	1 (1.72) (ND in CS)	1.000

Table 5 Chicken cloacal swabs (CS) and Breast meat (BM) AMR[1] EOH profiles from local slaughterhouses in Nakhon Ratchasima Province, Thailand (Cont.)

Sample	No. of AM[2]	No. of AM classes	Resistance phenotypic profiles	No. of isolates (%)	p value[3]
BM	6	4	AML-AMP-S-SXT-DO-OT	1 (1.72) (ND in CS)	1.000
BM	7	3	CL-FOX-AML-AMP-ENR-DO-OT	4 (6.90) (ND in CS)	0.120
BM	8	3	CL-FOX-CTX-AML-AMP-ENR-DO-OT	1 (1.72) (ND in CS)	1.000
BM	8	3	CL-FOX-CTX-AML-AMP-N-DO-OT	1 (1.72) (ND in CS)	1.000
BM	8	4	CL-FOX-AML-AMP-ENR-S-DO-OT	1 (1.72) (ND in CS)	1.000
BM	8	4	CL-AML-AMP-ENR-N-S-DO-OT	1 (1.72) (ND in CS)	1.000
BM	8	5	AML-AMP-C-ENR-N-S-DO-OT	1 (1.72) (ND in CS)	1.000
BM	9	5	CL-FOX-AML-AMP-CT-ENR-N-DO-OT	1 (1.72) (ND in CS)	1.000
Total MDR[6] *E. coli* O157:H7 isolates from cloacal swab samples				15 (25.86)	0.728
Total MDR[6] *E. coli* O157:H7 isolates from breast meat samples				17 (29.31)	

Note: AML=Amoxicillin, AMP=Ampicillin, C=Chloramphenicol, CL=Cephalexin, CT=Colistin, CTX=Cefotaxime, DO=Doxycycline, ENR=Enrofloxacin, FEP=Cefepime, FOX=Cefoxitin, N=Neomycin, OT=Oxytetracycline, S=Streptomycin, SXT=Sulfamethoxazole+ trimethoprim; [1]AMR=Antimicrobial resistance; [2]AM=Antimicrobial; [3]Statistical significance level was set at 95% confidence; AMR profile was compared if *E. coli* O157:H7 was detected in both cloacal swab and breast meat samples using the Chi square test. [4]CSBM=bacteria encountered in cloacal swab and breast meat samples; [4]ND=Not detected; [5]Multidrug resistance *E. coli* O157:H7; *Prevalence of AMR profile between cloacal swab and breast meat samples was significantly difference ($p < 0.05$)

Genotypes of antimicrobial resistance profiles of EOH isolated from cloacal swab and chicken breast meats.

The incidence of the ESBL-TEM gene from EOH isolates with MDR from samples was 65.38% while that of bla_{TEM} was 34.61%. The incidence of *int-1* gene of EOH with MDR was 3.85%. Moreover, the incidence of ESBL-TEM gene and bla_{TEM} and *int-1* of EOH with MDR isolated from cloacal swab samples was more than that from breast meat samples. The genotypic of AMR profiles of EOH is shown in Table 6.

Table 6 Prevalence of genes encoding AMR in EOH isolates from cloacal swabs and chicken breast meat from domestic poultry slaughterhouses in Nakhon Ratchasima Province, Thailand

Type of sample	ESBL-TEM gene	bla_{TEM} gene	*int-1* gene	*mcr-1* gene
Cloacal swab	9/13 (69.23%)	6/13 (46.15%)	1/13 (7.69%)	0/13 (0%)
Breast meat	8/13 (61.54%)	3/13 (23.08%)	0/13 (0%)	0/13 (0%)
p-value	1.000	0.411	1.000	N/A
Total	17/26 (65.38%)	9/26 (34.61%)	1/26 (3.85%)	0/26 (0%)

Note: N/A = not applicable. Data concerning about the relationship between the type of sample and the MDR status of bacteria were analyzed by using Chi-square tests with SPSS Statistics for Windows, Version 16.0; the significance level was set at 95% confidence interval ($p < 0.05$). *The prevalence of AMR genes between cloacal swab samples and breast meat samples was significantly different ($p < 0.05$).

DISCUSSION

E. coli is an indicator of the fecal contamination in food and water (Shecho et al., 2017; Elsharawy et al., 2022a; Elsharawy et al., 2022b). Moreover, EOH is dangerous to chicken meat consumers (Elsharawy et al., 2022a; Elsharawy et al., 2022b), and chicken intestines can contain a large number of pathogenic bacteria, especially EOH; therefore, chicken meat contaminated with EOH is a health concern for both animals and humans (Fuh et al., 2018). The current study indicates that the domestic poultry slaughterhouses in Nakhon Ratchasima Province have considerable problems in the slaughter process or other steps in the domestic poultry slaughterhouses. This agrees with the findings of (Elsharawy et al., 2022a) and (Elsharawy et al., 2022b) who reported that the high contaminations of *E. coli* in chicken meat indicate poor hygiene in slaughter processes, handling practices, transportation, or during meat processing in slaughterhouses because *E. coli* is found in the gastrointestinal tract of animals and humans. Nevertheless, the prevalence range of *E. coli* and EOH depends on the countries and reagents involved (Ranasinghe et al., 2022), method of sampling, detection of EOH method, level of contamination of animals, management of animals at farms and abattoirs (Anyanwu et al., 2022), and the type of samples (Anyanwu et al., 2022; Ranasinghe et al., 2022).

Thus, results in numerous studies that described the prevalence of EOH differed from the results of this study, such as with imported chicken meat (6.07%) in Saudi Arabia between 2017 (Alhadlaq et al., 2023), chicken meat (40%) in Saudi Arabia between 2021 to 2022 (Elsharawy et al., 2022a) and (Elsharawy et al., 2022b), cloacae swab of adult chicken (7.5%) and of young chicken (18.8%) in Ethiopia between 2015 to 2016 (Shecho et al., 2017), cloacal swab of chicken aged 1–3 weeks (10.89%) and chicken aged 4–12 weeks (5.36%) in Nigeria between 2017 and 2018 (Fuh et al., 2018), fecal sample of broiler chicken (1.29%) in Nigeria between 2021 and 2022 (Anyanwu et al., 2022), fecal sample of chickens (10%) in Nigeria in 2006 (Aibinu et al., 2007), broiler chicken farms (0%) in Canada between 2004 and 2005 (Diarrassouba et al., 2007), chickens (10.97%) in China between 1999 and 2000 (Zhu et al., 2023), chicken meat (3.2%) in China between 2012 and 2016 (Zhang et al., 2022), and chicken nuggets (0%) in Iran in 2013 (Miri et al., 2022). According to Elsharawy et al. (2022a), Elsharawy et al. (2022b), and Saleman et al. (2022) EOH transmission to humans occurs via contamination in chicken meat or drinking of contaminated water. Additionally, EOH contaminated meat through the slaughter process or chicken meat-production step (Saleman et al., 2022; Alhadlaq et al., 2023). Our study is the first report of EOH isolated from chickens and chicken meats at local poultry slaughterhouses in Nakhon Ratchasima Province, Thailand. Therefore, good manufacturing practices (GMP) and good hygienic practices (GHP) of poultry slaughterhouses are important and will support successful safe food production (Claire et al., 2013). Generally, AMR is a globally important health issue (Amin et al., 2020). A high rate of AMR and MDR of *E. coli* and EOH indicates that chickens have been exposed to those antimicrobial drugs (Anyanwu et al., 2022). Furthermore, tetracycline, AML, and AMP are normally used as veterinary antimicrobials for chickens and commercial poultry (Fuh et al., 2018; Ranasinghe et al., 2022), and bacteria in the intestine of chickens can consequently develop resistance to these antimicrobial drugs (Fuh et al., 2018) that are misused in chickens (Ranasinghe et al., 2022). Moreover, Shecho et al. (2017) reported that the high

level of resistance to erythromycin, AMP, and tetracycline of EOH isolated from cloacae swabs of chickens is because these antimicrobial drugs are often used with veterinary and human medicine.

Canada has prohibited tetracycline as a growth promoter in broilers, although *E. coli* isolated from chicken farms can exhibit high resistant to tetracycline-based drugs because these are used to treat *E. coli* infections in chickens (Diarrassouba et al., 2007). Furthermore, a frequent mechanism for tetracycline resistance of *E. coli* is because of removal of antibiotic from the bacterial cell via efflux pump for carrying drugs outside the cell (Diarrassouba et al., 2007).

Similarly, our study shows that the total of EOH with MDR is 5.73%. Furthermore, EOH isolated from chicken breast meats exhibited more MDR profiles than those from cloacal swab of chickens. MDR can be caused by many reasons, including the misuse of antimicrobial drugs (Ranasinghe et al., 2022) and is mediated by genetic mobile elements, such as plasmids, transposons, and integrons (Shecho et al., 2017). Moreover, *E. coli* strains exhibiting MDR can be transmitted to humans after consumption of chicken meat (Bhoomika et al., 2016). Therefore, the most effective protection against EOH with AMR and MDR is good hygiene via GMP and GHP in slaughterhouses and reasonable usage of the antimicrobial drugs in live chickens (Elsharawy et al., 2022a) and (Elsharawy et al., 2022b). One health approach is to reduce the use of antimicrobial drugs to avoid hazardous effect of antimicrobials drug on livestock and humans (Hosain et al., 2021). Additionally, houseflies from hospital or animal farm area transmit antimicrobial resistance bacteria among humans and animals (Akter et al., 2020). AMR in *E. coli* transmit to humans via wild animals (Asai et al., 2022). Additionally, houseflies at local poultry slaughterhouses may play an important role as vectors and reservoirs in antimicrobial resistant bacteria and antimicrobial resistance genes transmission among chickens, chicken meat, and humans (Usui et al., 2022). Moreover, AMR in bacteria is transmitted between animals, humans, and environments via food chains, such as those including vegetables, or via direct and indirect contact (Sobur et al., 2019).

The predominant antimicrobial resistance gene of EOH here was the ESBL-TEM gene as also reported by Hosain et al. (2021). In Thailand, ESBL-producing *E. coli* have been isolated from poultry (40%) and poultry meat (50%) because cephalosporins have been popularly used in clinical practice (Ranasinghe et al., 2022), and consequently, ESBL-producing *E. coli* strains have been transmitted from chicken meat to humans (Bhoomika et al., 2016). Moreover, several EOH strains harbor beta-lactamase genes but they did not exhibit an ESBL phenotype because they conceal the expression of ESBL phenotypic resistance (Aibinu et al., 2007). Houseflies in domestic poultry slaughterhouses may also play an important role as vectors and reservoirs in bacteria with AMR and genetic transmission of AMR among chickens, chicken meat, and humans (Usui et al., 2022).

Nakayama et al. (2022) reported that the high incidence of *mcr-1* in *E. coli* isolated from chicken meat sample indicated that this gene may be spread in chicken, and Gharaibeh et al. (2019) reported that *mcr* encodes colistin drug resistance can be transmitted horizontally among animals, humans, and environments via bacteria. The EOH isolates found in this study did not harbor *mcr-1* (0%), which implied that broiler farms have favorable biosecurity and that colistin drugs were not used in broilers. Moreover, this agrees with Nakayama et al. (2022) who reported that the incidence of *mcr-1* of *E. coli* isolated from chicken meat sample was 0% during 2021–2022 in Japan. Thus,

bacteria in domestic poultry slaughterhouses in Nakhon Ratchasima Province may not harbor *mcr-1* because this gene is transferred to EOH by conjugation (Zhang et al., 2022; Luo et al., 2023).

Similarly, the presence of integron genes in *E. coli* reflects that *E. coli* acts as a reservoir and antimicrobial gene spreader in food chains and the environment (Diarrassouba et al., 2007). Our study shows a very low rate of class 1 integrons in *E. coli*, and it is possible that these genes are of less concern as spreaders of AMR. However, bacteria exchange genes encoding AMR among animals, humans, and environments. Therefore, surveillance for antimicrobial resistance profile of EOH isolated from chickens and chicken meat is important for continued risk assessment of EOH. Our result implied that this problem of AMR may be from vectors and reservoirs because according to Veterinary Council of Thailand Organization (2020), the cephalosporin and quinolone group were prohibited from being used in poultry. Hence, the subsequent plan for controlling the spread of AMR bacteria at local poultry slaughterhouses should be focused on control vectors such as houseflies, cockroaches, insects, and meat hygiene of the poultry slaughterhouse process.

CONCLUSIONS

EOH was detected in cloacal swab samples (pre-slaughter) and chicken meat samples (post-slaughter) from domestic poultry slaughterhouses in Nakhon Ratchasima Province, Thailand and could be transmitted via genes encoding AMR among chickens and by chicken meat that could spread bacteria with AMR in the environment and to humans. Moreover, this research is the first report on the prevalence of *Escherichia coli* O157:H7 isolated from chickens and their meat products at local slaughterhouses in Nakhon Ratchasima Province, Thailand. Therefore, EOH should be monitored, and surveillance and risk analysis undertaken to protect against EOH contamination during slaughter and chicken meat process.

ACKNOWLEDGEMENTS

Department of Veterinary Public Health, Faculty of Veterinary Medicine, Khon Kaen University, and Agricultural Research Development Agency (Public Organization), Bangkok, Thailand. The study was supported by a grant from the Ph.D. Scholarship for Chalermprakiat 70 years of the reign from Agricultural Research Development Agency (Public Organization).

AUTHOR CONTRIBUTIONS

PN and PS: Conceptualized and designed the study. PN, TS, SL, and CN: Contributed to sample collection, microbiological culturing, antimicrobial susceptibility test, and PCR running. PN, CS, and PS: Performed statistical analyses. PN: Drafted the manuscript. PN: Revised the manuscript. All authors have read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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How to cite this article;

Pattarakitti Noenchat, Thanawan Srithong, Sujeeporn Lertpiriyasakulkit, Chawakorn Nhoonoi, Chuleeporn Saksangawong and Pairat Sornplang. Prevalence and antimicrobial resistance profile of Escherichia coli O157:H7 isolated from chickens and chicken meats from local poultry slaughterhouses in Nakhon Ratchasima Province, Thailand.Veterinary Integrative Sciences. 2024; 22(3): 769 - 785