

Multidrug resistance, extended-spectrum β -lactamase (ESBL) production, and colistin resistance in *Escherichia coli* from retail chicken meat in fresh markets in Bangkok, Thailand

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Abstract

Contamination of chicken meat with antimicrobial-resistant bacteria, particularly multidrug-resistant (MDR), colistin-resistant, and extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* (*E. coli*), threatens its safety for human consumption. This study was conducted to determine the prevalence and antimicrobial resistance (AMR) profiles, particularly ESBL production and colistin resistance in *E. coli* isolated from retail chicken meat in fresh markets in Bangkok, Thailand. A total of 200 (81.6%) *E. coli* isolates were recovered from 245 chicken meat samples collected from fresh markets across six regions of Bangkok. Among the isolates, 189 (94.5%) exhibited resistance to at least one antimicrobial agent tested, with the highest resistance observed for ampicillin (83.5%), followed by tetracycline (82.5%). Notably, 79.0% of the isolates were classified as MDR. Furthermore, ESBL production and colistin resistance were detected in 2% (n=4) and 4.5% (n=9) of the isolates, respectively. Among the AMR genes investigated, blaTEM-1 was the most frequently detected (61.5%, n=123), followed by mcr-1 (3%, n=6), blaCTX-M-14 (1%, n=2), blaCTX-M-55 (0.5%, n=1), and mcr-3 (0.5%, n=1). Multilocus sequence typing (MLST) analysis of ESBL-producing and colistin-resistant *E. coli* isolates (n=13) identified seven distinct sequence types (STs), with ST162 (n=2) and ST209 (n=2) being the most prevalent. These findings indicate that retail chicken meat may serve as a potential reservoir of MDR, colistin-resistant, and ESBL-producing *E. coli*, posing a potential risk of transmission to humans through the food chain. Strengthening surveillance and implementing a One Health approach are essential to mitigate this emerging public health threat.

Keywords: chicken meat, colistin resistance, ESBL, *Escherichia coli*, multidrug resistance, Thailand

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Introduction

The rapid development and dissemination of AMR have become major health threats globally in the twenty-first century. The World Health Organization (WHO) has prioritized AMR as one of the top ten global health threats to humanity (WHO, 2023). In 2019, bacterial AMR was linked to approximately 4.95 million human deaths worldwide. (Murray *et al.*, 2022). The Southeast Asia (SEA) region has been described as a major global epicenter for the development and dissemination of AMR (Zellweger *et al.*, 2017). Approximately 38,000 deaths annually are caused by AMR in Thailand alone, with an additional economic cost of US\$1.2 billion (Pumart *et al.*, 2012). If no effective measures are taken to address the threat of AMR, it is projected that by 2050, AMR could result in ten million fatalities each year and a cumulative economic cost of US\$100 trillion globally (O'Neill, 2016).

A specific concern regarding AMR is the rapid global spread of MDR bacteria, which are resistant to at least three classes of antimicrobials, and ESBL-producers, which are responsible for resistance to several β -lactam antibiotics (3rd- and 4th-generation cephalosporins) (Poirel *et al.*, 2018). MDR bacteria and ESBL-producers significantly limit the number of effective drugs available for treating infections. Carbapenem and colistin are regarded as the last-resort antibiotics for managing infections caused by MDR and ESBL-producing bacteria, particularly Enterobacteriaceae (Elmonir *et al.*, 2021). Nevertheless, the increasing use of these critical antibiotics in the last few years has led to worries about the emergence and transmission of resistance even to these last-line therapies among Enterobacteriaceae.

Escherichia coli (*E. coli*), a member of the family Enterobacteriaceae, is a commensal bacterium commonly found in the intestinal microbiota of humans and animals (Poirel *et al.*, 2018). As a commensal bacterium, it has an opportunity to be in close contact with numerous other bacteria throughout the gastrointestinal tract. This creates a chance for this bacterium to acquire genetic material, including AMR genes, from other bacteria and to transfer them to other bacteria, mostly through horizontal gene transfer (Puvača and de Llanos Frutos, 2021). Consequently, *E. coli* functions as a natural reservoir of resistance determinants that can potentially be disseminated to other bacteria (Poirel *et al.*, 2018). The increasing prevalence of antimicrobial-resistant *E. coli* strains poses a significant threat to human health, leading to increased rates of morbidity and mortality (de Been *et al.*, 2014).

The extensive application of antimicrobials in livestock production, involving the poultry industry, is a key driver of the development and transmission of AMR. Animals and their products are capable of acting as reservoirs for resistant bacteria, with the potential for AMR genetic determinants to be transmitted to humans via the food chain (Kim *et al.*, 2020). Chicken meat, a widely consumed animal product, is a notable reservoir for AMR bacteria, including *E. coli*. MDR *E. coli* is more frequently observed in chicken meat than

in other kinds of meat (Moawad *et al.*, 2018). Several studies have reported very high rates of MDR *E. coli* strains from poultry sources in Asia (Al Azad *et al.*, 2019; Rahman *et al.*, 2020). Furthermore, instances of colistin resistance in ESBL producers or carbapenem-resistant *E. coli* have been identified in chicken meat (Aklilu *et al.*, 2022).

In recent years, the prevalence of antimicrobial-resistant *E. coli* from human, animal, food, and environmental sources has increased worldwide (Pires *et al.*, 2022; Mandujano-Hernández *et al.*, 2024). The extensive and often indiscriminate use of antimicrobials in livestock production has further accelerated the emergence and spread of resistant bacteria, including MDR *E. coli*. Foods of animal origin, particularly chicken meat, may serve as reservoirs of these resistant organisms, thereby facilitating human exposure through the food chain. Therefore, understanding the prevalence and AMR patterns of MDR *E. coli* from chicken meat is crucial for public health.

Chicken meat is one of the most widely consumed types of meat in Thailand, and the country ranked as the fourth-largest exporter of chicken meat worldwide in 2020 (Department of Trade Negotiation, 2021; Sornsenee *et al.*, 2022). Bangkok, the political, economic, and commercial capital of Thailand, is also the country's most densely populated city (Edelman, 2022). This high population density, coupled with substantial chicken meat consumption, underscores the need for continuous surveillance of AMR *E. coli* in the chicken meat supply.

Chaisatit *et al.* (2012) previously reported a high prevalence of antimicrobial-resistant *E. coli* contamination in chicken meat obtained from supermarkets in Bangkok. More recently, the co-occurrence of ESBL production and colistin resistance in *E. coli* from animal, environmental, and human sources has been documented in Thailand (Runcharoen *et al.*, 2017; Lay *et al.*, 2021; Chotinantakul *et al.*, 2022). These findings highlight the persistent public health risk posed by colistin-resistant and ESBL-producing *E. coli* in Thailand.

Fresh markets serve as major retail sources of raw meat and vegetables in Thailand but may pose an elevated risk of bacterial contamination due to inconsistent hygienic practices (Ananchaipattana *et al.*, 2012). To our knowledge, limited studies have investigated ESBL-producing and colistin-resistant *E. coli* in chicken meat from fresh markets in Bangkok, Thailand. Therefore, this study aimed to determine the prevalence and AMR profiles of *E. coli* isolated from retail chicken meat obtained from fresh markets in Bangkok, Thailand, with particular emphasis on ESBL production and colistin resistance.

Materials and Methods

Sample size calculation: The total number of chicken meat samples required for this study was estimated using the sample size calculation formula described by Thrusfield (2018). Based on a previously reported *E. coli* prevalence of 36–85% in meat products in Thailand (Trongjit *et al.*, 2016), the sample size was calculated

using an expected prevalence of 85% (0.85) in chicken meat, with a 95% confidence level and a desired precision of 5% (0.05).

Where, n = the number of bacterial isolates required for susceptibility testing,

P_{exp} = the expected prevalence of resistant isolates (0.5),

e = the desired absolute precision (0.05).

This calculation yielded a minimum sample size of 196 chicken meat samples. To increase statistical robustness, a total of 245 samples were collected and included in this study.

Sample collection: A cross-sectional study was conducted to investigate *E. coli* contamination and AMR in retail chicken meat samples collected from fresh markets located in six regions of Bangkok, Thailand (Central Bangkok, Southern Bangkok, Northern Bangkok, Eastern Bangkok, North Thonburi Bangkok and South Thonburi Bangkok) (Fig. 1). From each region, two fresh markets were randomly selected based on the distribution of markets, and at least 20 chicken meat samples were collected from each market (Table 1).

The types of chicken meat cuts collected in this study included breast (fillet, brisket), leg quarter (drumstick, thigh), and wing (drumette, wingette, wing tip) (Table 2). These cuts were selected to represent commonly consumed parts and to assess potential variation in microbial contamination across different meat types. All meat samples were

individually placed in labeled zip-lock polyethylene bags, maintained at 4 °C on ice, transported to the Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University, Thailand, and processed within 8 hours of collection.

***E. coli* isolation:** All chicken meat samples ($n=245$) were analyzed for the isolation and identification of *E. coli* following the standard protocol outlined in the Bacteriological Analytical Manual (BAM) of the U.S. Food and Drug Administration (U.S. FDA) (Feng *et al.*, 2020). Briefly, 225 mL of buffered peptone water (BPW) (Difco, MD, USA) was mixed with 25 g of chicken meat samples and well homogenized in a stomacher machine (Seward Stomacher® 400 Circulator, UK) at 230 rpm for 2 minutes. Approximately 1 mL of the homogenate was transferred into 9 mL of lactose broth (Difco, MD, USA) containing a Durham tube. Turbidity and gas production in the Durham tube were examined after incubation at 37°C for 18 to 24 hours. One loopful from each gas-positive tube was inoculated into 9 mL of EC broth (Difco, MD, USA) containing a Durham tube and incubated at 44.5°C in a water bath. After overnight incubation, a loopful from each gas-positive EC broth culture was streaked onto Levine's eosin-methylene blue (L-EMB) agar (Difco, MD, USA). Following overnight incubation at 37°C, colonies exhibiting a green metallic sheen with dark centers on L-EMB agar were considered presumptive *E. coli* and confirmed by biochemical tests, including indole production, methyl red, Voges-Proskauer, and citrate utilization (IMViC) tests.

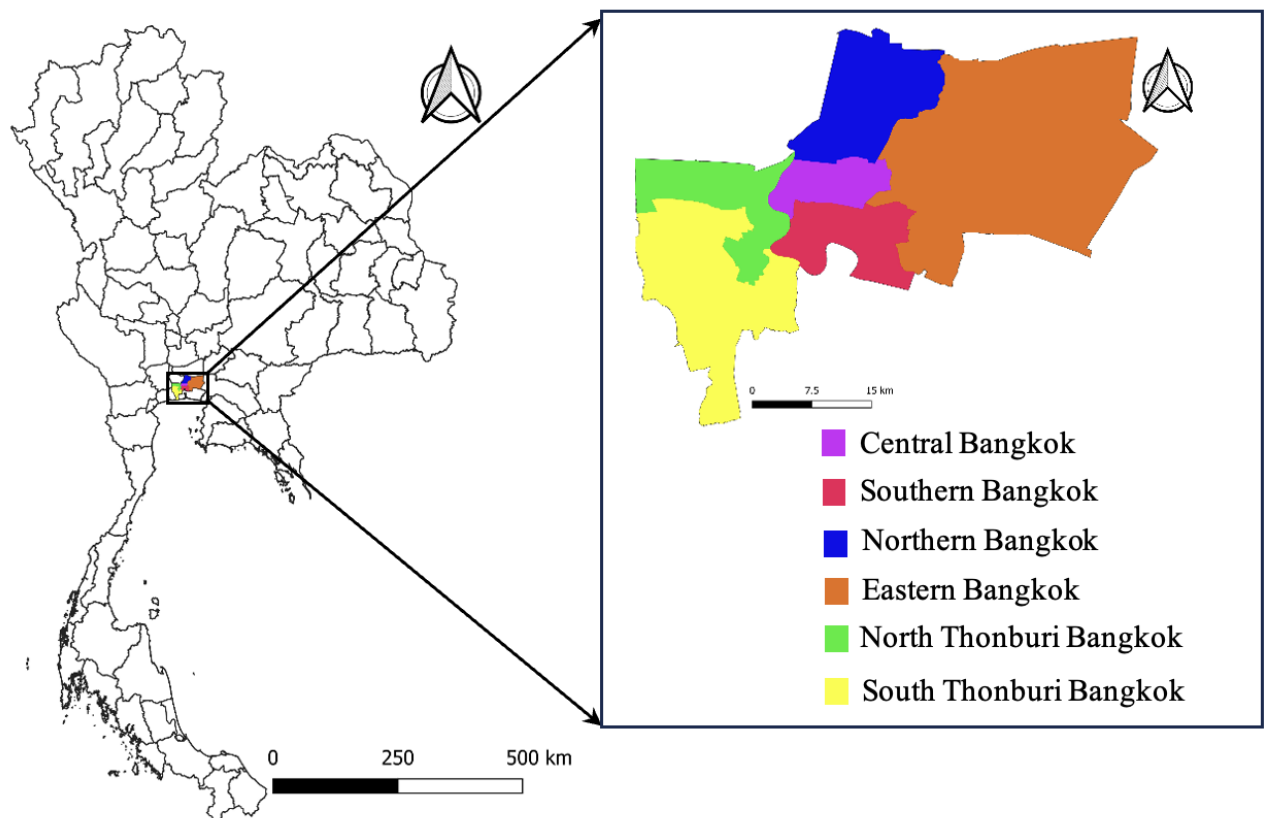


Figure 1 Location of the study area (six regions of Bangkok).

Table 1 The number of chicken meat samples collected from six regions of Bangkok.

Region	No. of chicken meat samples
Central Bangkok	41
Southern Bangkok	41
Northern Bangkok	41
Eastern Bangkok	40
North Thonburi Bangkok	41
South Thonburi Bangkok	41
Total	245

Table 2 The number of chicken meat samples stratified by meat types.

Meat types	No. of chicken meat samples
Breast	87
Leg quarter	95
Wing	63
Total	245

Antimicrobial susceptibility testing: The antimicrobial susceptibility profiles of all isolated *E. coli* strains from chicken meat samples were assessed against nine antibiotics: ampicillin (AMP), chloramphenicol (CHL), ciprofloxacin (CIP), colistin (COL), gentamicin (GEN), streptomycin (STR), sulfamethoxazole (SUL), tetracycline (TET), and trimethoprim (TMP), using the two-fold agar dilution method in accordance with protocols established by the Clinical and Laboratory Standards Institute (CLSI) VET01 (6th edition, Chapter 6) (CLSI, 2024a). These antibiotics were selected based on their frequent use and clinical importance in both human and veterinary medicine.

Quality control for susceptibility testing was performed using *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853. Isolates were classified as susceptible or resistant based on clinical breakpoints according to CLSI M100 (34th edition, Table 2A-1) (CLSI, 2024b) and the National Antimicrobial Resistance Monitoring System (NARMS, 2024) for the antibiotic streptomycin. Isolates showing resistance to at least three different antimicrobial classes of tested antibiotics were identified as MDR.

Detection of ESBLs and carbapenemase production: Initial screening for ESBL and carbapenemase production was performed using the standard disk diffusion method according to the procedures described in CLSI VET01 (6th edition, Chapter 4) (CLSI, 2024a). Three cephalosporin antibiotic disks, ceftazidime (CAZ, 30 µg), cefotaxime (CTX, 30 µg), and cefpodoxime (CPD, 10 µg), were used for ESBL screening, while meropenem (MEM, 10 µg) disks were used for carbapenemase screening. All antibiotic disks were obtained from Oxoid Ltd. (Basingstoke, UK). Isolates were categorized as susceptible or resistant according to the zone diameter breakpoints specified by CLSI M100 (34th edition, Table 2A-1) (CLSI, 2024b).

Isolates exhibiting resistance to at least one of the tested cephalosporins were confirmed as ESBL producers using the combination disk test following CLSI M100 (34th edition) (CLSI, 2024b). All meropenem-resistant *E. coli* isolates were considered potential carbapenemase producers and were further confirmed using the modified carbapenem inactivation

method (mCIM), as described by CLSI M100 (34th edition) (CLSI, 2024b). Quality control for antimicrobial susceptibility testing was performed using *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

Determination of multiple antibiotic resistance (MAR) index: The multiple antibiotic resistance (MAR) index for each *E. coli* isolate was determined using the formula $MAR = a/b$, where “a” is the number of antibiotics to which the isolate was resistant and “b” is the total number of antibiotics tested for susceptibility (Krumperman, 1983).

Selected AMR gene detection: Polymerase chain reaction (PCR) was performed to detect the selected AMR genes in all *E. coli* isolates recovered in this study. The extraction of bacterial DNA was performed using the whole-cell boiling method, as previously described by Levesque *et al.* (1995). Genes encoding β-lactamase (blaTEM, blaSHV, and blaCTX-M groups), carbapenemases (blaIMP, blaVIM, blaOXA, blaNDM-1 and blaKPC), and plasmid-mediated colistin resistance (mcr-1 to mcr-10) were amplified by singleplex PCR (sPCR) or multiplex PCR (mPCR) using primer sets and protocols listed in Table 3.

Amplification was conducted in a 25 µl PCR reaction mixture including 1 µl of DNA template, 0.5 µl of dNTP mixture (200 µM of each dNTP) (TaKaRa BIO, Shiga, Japan), 1 µl of each 10 µM forward and reverse primer (0.5 µl each in mPCR), 2.5 µl of 10× PCR buffer, 0.125 µl of HotStarTaq® DNA Polymerase (Qiagen, Hilden, Germany) and nuclease-free water to reach the final volume. PCR amplifications were performed in a thermal cycler (Biometra, Göttingen, Germany) and products were separated on a 1.5% (w/v) agarose gel (1st BASE, Singapore), stained with RedSafe™ Nucleic Acid Staining Solution (iNtRON Biotechnology, Seongnam, South Korea). The results were then visualized under UV light with the Omega Fluor™ Gel Documentation System (Aplegen, Pleasanton, CA, USA).

The PCR products from isolates carrying resistance genes were subjected to Sanger sequencing to confirm amplification of the target genes. The PCR products were cleaned following the manufacturer's protocol

using the NucleoSpin® Gel and PCR Clean-up kit (MACHEREY-NAGEL, Düren, Germany), and subsequently sequenced (Bionics Co., LTD., Gyeonggi-Do, Republic of Korea). Obtained nucleotide sequences were trimmed to remove low-quality ends, and consensus sequences were generated by BioEdit software (version 7.7.1)(Hall, 1999). The sequences were then compared with reference sequences available in the GenBank database through the BLAST tool offered by the National Center for Biotechnology Information (NCBI) (NCBI, 2024).

Multilocus sequence typing (MLST): All ESBL-producing and colistin-resistant *E. coli* isolates in this study were subjected to MLST analysis. MLST was performed by PCR amplification of seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) of the *E. coli* isolates using the primers and protocol as described by Tartof *et al.* (2005) (Table 4). The amplified PCR products were purified with the NucleoSpin® Gel and PCR Clean-up Kit (MACHEREY-NAGEL, Düren, Germany) and then sent for Sanger sequencing (Bionics Co., Ltd., Gyeonggi-Do, Republic of Korea). The resulting sequences were submitted to the pubMLST database (https://pubmlst.org/bigdb?db=pubmlst_escherichia_seqdef) to determine the allelic profiles of the housekeeping genes. Sequence types (STs) of *E. coli* isolates were assigned based on identical sequences at all seven loci in the database, while isolates without exact matches were designated as unknown STs (UNT).

Statistical analysis: Statistical analysis was performed to assess the prevalence and AMR patterns of *E. coli* using descriptive data analysis methods. The prevalence of *E. coli* in chicken meat samples across six different regions and among three different types of meat was compared using Pearson's Chi-square test. The mode and range of MICs were compared between resistant and susceptible *E. coli* isolates.

Logistic regression analysis was performed to assess associations among resistance phenotypes and MDR characteristics. The variable with the highest resistance rate was considered the dependent variable. Univariate analysis was conducted to screen for potentially significant predictors ($P < 0.1$), followed by a stepwise approach using forward selection and backward elimination. Statistically significant associations between dependent and independent variables were determined based on p-values ($P < 0.05$) and likelihood ratio tests in the final analysis. All statistical analyses were performed using SPSS statistical software, version 29 (IBM Corp., Armonk, NY, USA).

Results

E. coli prevalence in chicken meat samples: In this study, 81.6% (200/245) of chicken meat samples were contaminated with *E. coli*. The prevalence of *E. coli* did not differ significantly among the six regions of Bangkok ($P > 0.05$). The highest prevalence was observed in Southern Bangkok (92.7%, 38/41), followed by South Thonburi (90.2%, 37/41), Northern Bangkok (80.5%, 33/41), North Thonburi (78.0%, 32/41), Eastern Bangkok (75.0%, 30/40), and Central Bangkok (73.2%, 30/41).

Similarly, no statistically significant difference in *E. coli* prevalence was observed among different meat types ($P > 0.05$). The highest prevalence was detected in breast samples (83.9%, 78/93), followed by leg quarters (83.1%, 74/89) and wings (76.2%, 48/63).

Antimicrobial resistance in *E. coli* isolates: Of the 200 *E. coli* isolates recovered from retail chicken meat samples, 94.5% (189/200) were resistant to at least one of the antibiotics tested. Overall, 79.0% (158/200) of the *E. coli* isolates in this study were MDR.

The highest resistance rate was observed for ampicillin (83.5%, 167/200), followed by tetracycline (82.5%, 165/200), whereas all isolates remained susceptible to ceftazidime and meropenem (Table 5). Notably, 4.5% (9/200) of the *E. coli* isolates were resistant to colistin, a last-resort antibiotic. All colistin-resistant isolates were MDR isolates.

A total of 82 resistance patterns were identified among the *E. coli* isolates in this study, of which AMP-CHL-CIP-STR-SUL-TET-TMP (7%) was the most common resistance profile (Table 6).

ESBLs and carbapenemase production in *E. coli* isolates: During the initial screening for ESBL production, 9 isolates (4.5%) were resistant to cefpodoxime, and 6 isolates (3.0%) were resistant to cefotaxime, whereas all isolates remained susceptible to ceftazidime. Among these cephalosporin-resistant isolates, 4 isolates (2.0%) were confirmed as ESBL producers, and all ESBL-producing *E. coli* isolates exhibited MDR. In addition, all *E. coli* isolates in this study were susceptible to meropenem, suggesting no phenotypic evidence of carbapenemase production.

Multiple antibiotic resistance (MAR) index: The MAR indices of all *E. coli* isolates ranged from 0 to 0.769, with an overall mean MAR index of 0.36. (Table 6). Among the isolates in this study, 79.5% (159/200) exhibited MAR indices greater than 0.2.

MIC frequency distribution of *E. coli* isolates from chicken meat: The MIC distribution pattern of *E. coli* isolates is presented in Figure 2. The mode MIC values for resistant isolates were at least two-fold higher than the clinical breakpoints for all antibiotics tested. Additionally, the mode MIC values for sulfamethoxazole among resistant isolates exceeded the upper detection limit ($>2048 \mu\text{g/mL}$) in this study.

Table 3 Primer sequences used for detection of AMR genes in this study.

Target gene	Sequence (5'-3')	PCR condition	Amplicon (bp)	Reference
Genes encoding β -lactamase (sPCR)				
<i>bla</i> _{TEM}	F: GCGGAACCCCTATTG R: TCTAAAGTATATATGAGTAAACTTGGTCTGAC	94°C, 60s; 50°C, 60s; 72°C, 60s (30 cycles)	964	(Olesen et al., 2004)
<i>bla</i> _{SHV}	F: TTCGCCTGTGTATTATCTCCCTG R: TTAGCGTTGCCAGTGYTCG	94°C, 60s; 50°C, 60s; 72°C, 60s (30 cycles)	854	(Hasman et al., 2005)
<i>bla</i> _{CTX-M}	F: CGATGTGCAGTACCAGTAA R: AGTGACCAGAATCAGCGG	94°C, 60s; 60°C, 60s; 72°C, 60s (30 cycles)	585	(Batchelor et al., 2005)
<i>bla</i> _{CTX-M} group-1	F: TTAGGAARTGTGCCGCTGYA R: CGATATCGTTGGTGGTRCCAT	94°C, 40s; 60°C, 40s; 72°C, 60s (30 cycles)	688	(Dallenne et al., 2010)
<i>bla</i> _{CTX-M} group-2	F: CGTTAACGGCACGATGAC R: CGATATCGTTGGTGGTRCCAT	94°C, 40s; 60°C, 40s; 72°C 60s (30 cycles)	404	(Dallenne et al., 2010)
<i>bla</i> _{CTX-M} group-8/25	F: AACRCRCAGACGCTCTAC R: TCGAGCCGGAASGTIGTYAT	94°C, 40s; 60°C, 40s; 72°C, 60s (30 cycles)	326	(Dallenne et al., 2010)
Genes encoding β -lactamase (sPCR)				
<i>bla</i> _{CTX-M} group-9	F: GTGACAAAGAGAGTGCAACGG R: ATGATTCTCGCCGCTGAAGCC	94°C, 60s; 55°C, 60s; 72°C, 60s (35 cycles)	850	(Sabaté et al., 2000)
<i>bla</i> _{CTX-M} group-15	F: GTGACAAAGAGAGTGCAACGG R: ATGATTCTCGCCGCTGAAGCC	94°C, 60s; 55°C, 45s; 72°C, 60s (35 cycles)	876	(Muzaheed et al., 2008)
Genes encoding carbapenemases (mPCR)				
<i>bla</i> _{IMP}	F: GGAATAGAGTGGCTTAAYTCTC R: GGTTTAAYAAAACAACCACC		232	(Poirel et al., 2011)
<i>bla</i> _{NDM}	F: GGTTTGGCGATCTGGTTTTC R: CGGAATGGCTCATCACGATC	95°C, 60s; 52°C, 60s; 72°C 60s (35 cycles)	621	(Poirel et al., 2011)
<i>bla</i> _{VIM}	F: GATGGTGTITGGTCGCATA R: CGAATGCGCAGCACCAG		390	(Poirel et al., 2011)
Genes encoding carbapenemases (mPCR)				
<i>bla</i> _{OXA-48}	F: GCGTGGTTAAGGATGAACAC R: CATCAAGTTCAACCCAACCG	95°C, 60s; 52°C, 60s; 72°C 60s (35 cycles)	477	(Poirel et al., 2011)
<i>bla</i> _{KPC}	F: CGTCTAGTTCTGCTGCTTG R: CTTGTCATCCTTGTTAGGCG		798	(Poirel et al., 2011)
Plasmid-mediated colistin resistance genes (mPCR)				
<i>mcr-1</i>	F: TCCAAAAATGCCCTACAGACC R: GCCACCACAGGCAGTAAAAAT		205	(Liu et al., 2020a)
<i>mcr-2</i>	F: CCTTTTGTGCTGATGGGTTT R: ATTTTGAGCATGGTGGTGT	94°C, 60s; 59°C, 60s; 72°C, 60s (35 cycles)	279	(Liu et al., 2020a)
<i>mcr-3</i>	F: CTGTCTGAACCAATCCCATT R: CCATCGTTCTCCTTCCAAAA		347	(Liu et al., 2020a)
Plasmid-mediated colistin resistance genes (mPCR)				
<i>mcr-4</i>	F: GATCCGAAGCTGTGTTCTG R: GCCAGCATTTGGTACGCTAGT		426	(Liu et al., 2020a)
<i>mcr-5</i>	F: GGTGGCCGAGAAGATAACA R: ATGTTGCCAGAAGGTCCAAC		522	(Liu et al., 2020a)
<i>mcr-6</i>	F: AGCTATGTCAATCCCGTGAT R: ATTGGCTAGGTTGTCAATC		252	(Borowiak et al., 2020)
<i>mcr-7</i>	F: GTCAGTTACGCCATGCTCAA R: TTCTGTGCGAGAAGTGTGG	94°C, 60s; 59°C, 60s; 72°C, 60s (35 cycles)	791	(Liu et al., 2020a)
<i>mcr-8</i>	F: AAAGTGAACCCGGTACAACG R: GCCATAGCACCTCAACACCT		943	(Liu et al., 2020a)
<i>mcr-9</i>	F: GCGGTTGTAAAGCGTATGT R: CAAATCGCGGTCAGGATTAT		635	(Liu et al., 2020a)
<i>mcr-10</i>	F: TATCCTGAGCCGTCTTGAAC R: GGATCAGCGAAGCGAGCAT		386	(Göpel et al., 2023)

Table 4 Primer sequences used for MLST analysis.

Target gene	Sequence (5'-3')	PCR condition	Amplicon (bp)	Reference
<i>adk</i>	ATTCTGCTTGGCGCTCCGGG CCGTCAACTTTCGCGTATTT	94°C, 60s; 54°C, 60s; 72°C, 60s (30 cycles)	583	(Tartof et al., 2005)
<i>fumC</i>	TCACAGGTCGCCAGCGCTTC GTACGCAGCGAAAAAGATTC	94°C, 60s; 54°C, 60s; 72°C, 60s (30 cycles)	806	(Tartof et al., 2005)
<i>gyrB</i>	TCGGCGACACGGATGACGGC GTCCATGTAGGCGTTCAGGG	94°C, 60s; 58°C, 60s; 72°C, 60s (30 cycles)	911	(Tartof et al., 2005)
<i>icd</i>	ATGGAAAAGTAAAGTAGTTGTTCCGGCACA GGACGCAGCAGGATCTGTT	94°C, 40s; 54°C, 60s; 72°C, 60s (30 cycles)	878	(Tartof et al., 2005)
<i>mdh</i>	ATGAAAGTCGCAGTCCTCGGCGCTGCTGGCG G TTAACGAACTCCTGCCCCAGAGCGATATCTTT CTT	94°C, 40s; 60°C, 60s; 72°C, 60s (30 cycles)	932	(Tartof et al., 2005)
<i>purA</i>	CGCGCTGATGAAAGAGATGA CATACGGTAAGCCACGCAGA	94°C, 40s; 54°C, 60s; 72°C 60s (30 cycles)	816	(Tartof et al., 2005)
<i>recA</i>	CGCATTCGCTTTACCCTGACC TCGTGCAAATCTACGACCGGA	94°C, 60s; 58°C, 60s; 72°C, 60s (35 cycles)	780	(Tartof et al., 2005)

Table 5 Antimicrobial resistance in *E. coli* isolates.

Antibiotics	No. of resistant isolates (%)
AMP	167 (83.5)
TET	165 (82.5)
STR	104 (52.0)
SUL	129 (64.5)
TMP	103 (51.5)
CIP	86 (43.0)
CHL	74 (37.0)
GEN	28 (14.0)
CPD*	9 (4.5)
COL	9 (4.5)
CTX*	6 (3.0)
CAZ*	0 (0.0)
MEM*	0 (0.0)

*Detected by disk diffusion method.

Table 6 AMR pattern of *E. coli* isolated from chicken meat (n=200).

AMR pattern	No. of isolates (%)	MAR index
Susceptible	11 (5.5)	0.000
AMP	3 (1.5)	0.077
CIP	1 (0.5)	0.077
SUL	3 (1.5)	0.077
TET	4 (1.5)	0.077
TMP	1 (0.5)	0.077
AMP-CIP	1 (0.5)	0.154
AMP-STR	1 (0.5)	0.154
AMP-SUL	3 (1.5)	0.154
AMP-TET	11 (5.5)	0.154
SUL-TET	1 (0.5)	0.154
SUL-TMP	1 (0.5)	0.154
AMP-CHL-TET	1 (0.5)	0.231
AMP-CIP-STR	1 (0.5)	0.231
AMP-CIP-TET	7 (3.5)	0.231
AMP-COL-TET	1 (0.5)	0.231
AMP-GEN-TET	1 (0.5)	0.231
AMP-STR-TET	5 (2.5)	0.231
AMP-SUL-TET	4 (2.0)	0.231
AMP-SUL-TMP	1 (0.5)	0.231
AMP-TET-TMP	3 (1.5)	0.231
CHL-CIP-STR	1 (0.5)	0.231
CIP-STR-SUL	1 (0.5)	0.231
CIP-SUL-TET	1 (0.5)	0.231
CIP-TET-TMP	1 (0.5)	0.231
AMP-CHL-CIP-TET	2 (1.0)	0.308
AMP-CHL-STR-TET	1 (0.5)	0.308
AMP-CHL-SUL-TET	6 (3.0)	0.308
AMP-CHL-TET-TMP	1 (0.5)	0.308
AMP-CIP-STR-TET	1 (0.5)	0.308

Table 6 (Continue)

AMR pattern	No. of isolates (%)	MAR index
AMP-CIP-SUL-TET	3 (1.5)	0.308
AMP-GEN-STR-SUL	1 (0.5)	0.308
AMP-GEN-STR-TET	1 (0.5)	0.308
AMP-GEN-TET-TMP	1 (0.5)	0.308
AMP-STR-SUL-TET	2 (1.0)	0.308
AMP-STR-SUL-TMP	2 (1.0)	0.308
AMP-STR-TET-TMP	1 (0.5)	0.308
AMP-SUL-TET-TMP	4 (4.0)	0.308
CHL-SUL-TET-TMP	2 (1.0)	0.308
STR-SUL-TET-TMP	1 (0.5)	0.308
AMP-CHL-CIP-SUL-TET	2 (1.0)	0.385
AMP-CHL-CIP-SUL-TMP	1 (0.5)	0.385
AMP-CHL-CIP-TET-TMP	1 (0.5)	0.385
AMP-CHL-COL-TET-TMP	1 (0.5)	0.385
AMP-CHL-GEN-TET-TMP	1 (0.5)	0.385
AMP-CHL-STR-SUL-TET	1 (0.5)	0.385
AMP-CHL-STR-TET-TMP	2 (1.0)	0.385
AMP-CIP-GEN-STR-TET	1 (0.5)	0.385
AMP-CIP-STR-SUL-TET	6 (3.0)	0.385
AMP-CIP-STR-TET-TMP	1 (0.5)	0.385
AMP-CIP-SUL-TET-TMP	2 (1.0)	0.385
AMP-CPD-CTX-STR-TET	1 (0.5)	0.385
AMP-CPD-CTX-SUL-TET	1 (0.5)	0.385
AMP-GEN-STR-SUL-TMP	1 (0.5)	0.385
AMP-GEN-SUL-TET-TMP	1 (0.5)	0.385
AMP-STR-SUL-TET-TMP	9 (4.5)	0.385
CHL-CIP-STR-SUL-TET	1 (0.5)	0.385
CHL-CIP-SUL-TET-TMP	1 (0.5)	0.385
CHL-STR-SUL-TET-TMP	1 (0.5)	0.385
CHL-SUL-STR-TET-TMP	1 (0.5)	0.385
AMP-CHL-CIP-GEN-SUL-TMP	1 (0.5)	0.462
AMP-CHL-CIP-STR-SUL-TET	2 (1.0)	0.462
AMP-CHL-CIP-SUL-TET-TMP	7 (3.5)	0.462
AMP-CHL-GEN-SUL-TET-TMP	2 (1.0)	0.462
AMP-CHL-STR-SUL-TET-TMP	4 (2.0)	0.462
AMP-CIP-GEN-STR-TET-TMP	1 (0.5)	0.462
AMP-CIP-STR-SUL-TET-TMP	8 (4.0)	0.462
AMP-COL-STR-SUL-TET-TMP	2 (1.0)	0.462
AMP-GEN-STR-SUL-TET-TMP	2 (1.0)	0.462
AMP-CHL-CIP-STR-SUL-TET-TMP	14 (7.0)	0.538
AMP-CHL-GEN-STR-SUL-TET-TMP	3 (1.5)	0.538
AMP-CIP-COL-STR-SUL-TET-TMP	1 (0.5)	0.538
AMP-CIP-CPD-STR-SUL-TET-TMP	3 (1.5)	0.538
AMP-CIP-GEN-STR-SUL-TET-TMP	3 (1.5)	0.538
AMP-CHL-CIP-COL-GEN-STR-SUL-TET	1 (0.5)	0.615
AMP-CHL-CIP-COL-STR-SUL-TET-TMP	1 (0.5)	0.615
AMP-CHL-CIP-CPD-CTX-STR-SUL-TET	2 (1.0)	0.615
AMP-CHL-CIP-GEN-STR-SUL-TET-TMP	3 (1.5)	0.615
AMP-CHL-CIP-COL-GEN-STR-SUL-TET-TMP	2 (1.0)	0.692
AMP-CHL-CPD-CTX-GEN-STR-SUL-TET-TMP	1 (0.5)	0.692
AMP-CHL-CIP-CPD-CTX-GEN-STR-SUL-TET-TMP	1 (0.5)	0.769

Multivariable logistic regression analysis of *E. coli* isolates: Ampicillin resistance, the most common resistance phenotype among *E. coli* isolates in this study, was selected as the dependent variable, while resistance to other antimicrobial agents and MDR status were included as independent variables.

Initial univariate analysis identified several variables with potential associations with ampicillin resistance ($P < 0.1$) (Table 7). These variables were subsequently included in a stepwise multivariable logistic regression model using both forward selection and backward elimination approaches.

In the final model, tetracycline resistance and MDR status remained significantly associated with ampicillin resistance (Table 8). Tetracycline resistance

was significantly associated with ampicillin resistance (OR = 0.235, 95% CI: 0.082–0.674, $P < 0.05$). Similarly, MDR status was significantly associated with ampicillin resistance (OR = 0.145, 95% CI: 0.052–0.404, $P < 0.001$).

No significant associations were observed between ampicillin resistance and resistance to chloramphenicol, ciprofloxacin, streptomycin, sulfamethoxazole, or trimethoprim in the final model.

Presence of selected AMR genes in *E. coli* isolates: Among the selected AMR genes tested, blaTEM (61.5%, 123/200), blaCTX-M groups (1.5%, 3/200), mcr-1 (3.0%, 6/200), and mcr-3 (0.5%, 1/200) were detected in this study. Sequence analysis revealed that all blaTEM genes were identified as blaTEM-1, while two

blaCTX-M genes were identified as blaCTX-M-14 and one as blaCTX-M-55. All blaCTX-M and mcr genes harboring *E. coli* isolates exhibited phenotypic ESBL production and colistin resistance, respectively.

Multilocus sequence typing of *E. coli* isolates: MLST analysis of ESBL-producing and colistin-resistant *E.*

coli isolates revealed seven distinct sequence types (STs) among nine isolates, whereas four isolates were untypeable (ST-UNT) (Fig. 3). ST162 and ST209 were the predominant STs (n=2 each), while ST48, ST10, ST297, ST761, and ST2197 were each identified in a single isolate.

Table 7 Univariate analysis of the association between *E. coli* resistance to ampicillin and resistance to other antibiotics.

Predictors	Odds ratio	Std. Err.*	95% C.I.**	P-value
CHL	0.412	0.454	0.169 – 1.004	0.051
CIP	0.300	0.453	0.123 – 0.729	0.008
STR	0.181	0.477	0.071 – 0.462	<0.001
SUL	0.333	0.390	0.155 – 0.715	0.005
TET	0.078	0.440	0.033 – 0.185	<0.001
TMP	0.291	0.421	0.128 – 0.664	0.003
MDR	0.068	0.440	0.029 – 0.161	<0.001

*Std. Err. is Standard Error and **C.I. is Confidence Interval.

Table 8 Final logistic regression model showing the association between *E. coli* resistance to ampicillin and resistance to other antibiotics.

Predictors	Odds ratio	Std. Err.*	95% C.I.**	P-value
TET	0.235	0.538	0.082 – 0.674	0.007
MDR	0.145	0.524	0.052 – 0.404	<0.001
Constant	15.456	0.326	-	<0.001

*Std. Err. is Standard Error, and **C.I. is Confidence Interval.

		MIC range (µg/ml)																		
		≤0.0156	0.0313	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Antibiotics	AMP					0	0	0	10	18	3	2	0	3	18	70	61	15		
	CHL							0	38	58	20	10	24	13	27	10	0	0		
	CIP	28	5	2	10	41	28	13	10	9	26	16	12	0						
	COL			0	0	1	138	52	0	0	7	2	0							
	GEN				20	32	30	85	5	0	0	2	2	12	9	2	1			
	STR								0	15	52	29	17	26	19	19	13	10		
	SUL							0	0	0	2	4	46	12	7	0	0	0	8	121
	TET				0	0	3	26	4	2	0	3	25	71	54	12				
	TMP				59	30	8	0	0	0	0	0	0	0	0	2	51	50		

Figure 2 MIC frequency distribution of *E. coli* isolates from chicken meat (n=200). Dark red vertical lines indicate the clinical breakpoints for each antibiotic tested. Light blue and light green represent the susceptible and resistant ranges, respectively. Blue indicates the mode (highest frequency) of susceptible *E. coli* isolates, while green represents the mode of resistant isolates.

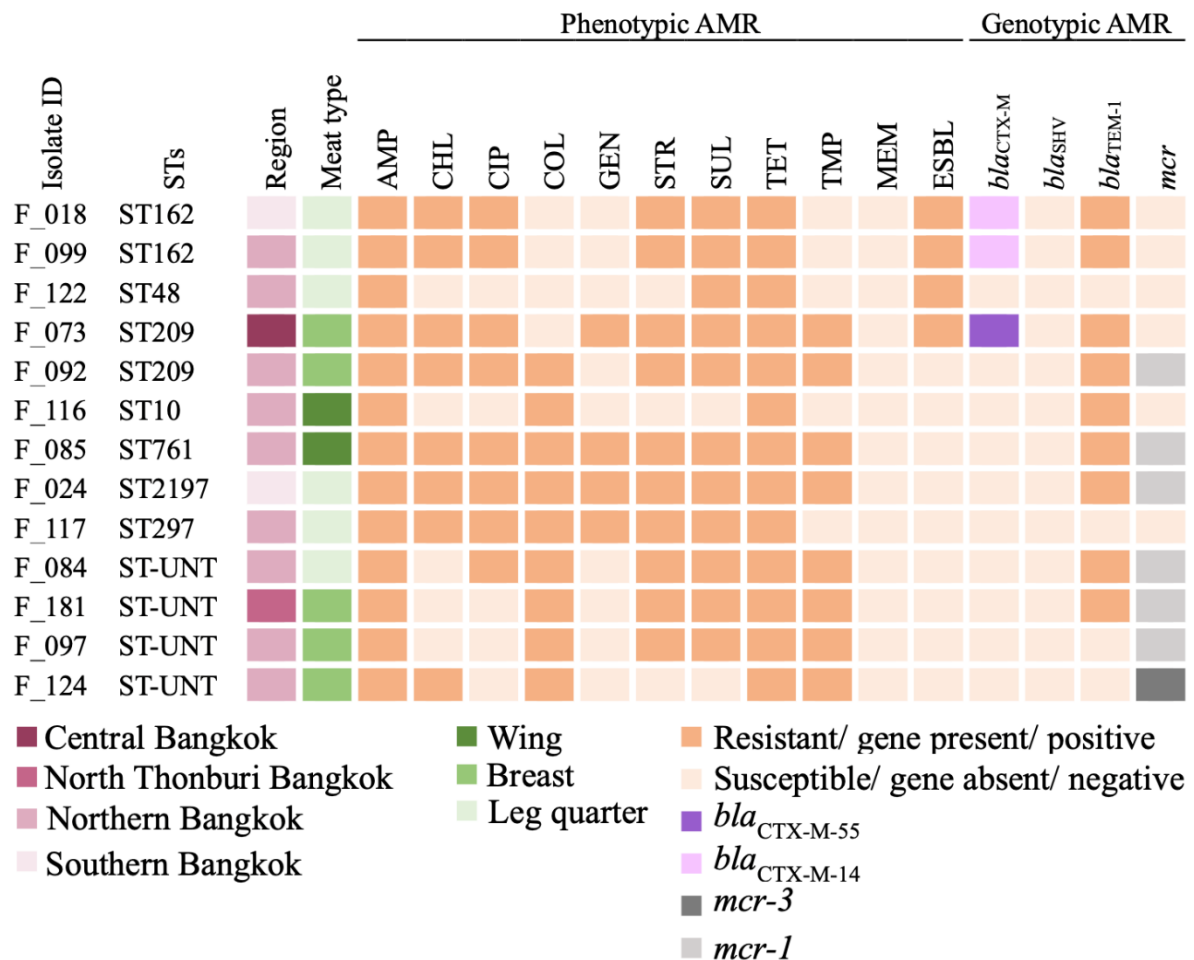


Figure 3 MLST profiles of ESBL-producing and colistin-resistant *E. coli* isolates (n=13).

Discussion

E. coli is commonly used as a microbial indicator to assess hygiene practices throughout the meat processing chain (Al-Mazrouei *et al.*, 2024). In this study, 81.6% of the collected chicken meat samples were contaminated with *E. coli*, which is consistent with a previous report showing a prevalence ranging from 36% to 85% in pig and chicken meat samples collected from slaughterhouses and fresh markets in Thailand (Trongjit *et al.*, 2016). The gastrointestinal tract, skin, and feathers of chickens, as well as the slaughterhouse environments, harbor diverse microbiota, including *E. coli*, which can contaminate carcasses and meat cuts under poor hygienic conditions during slaughtering (Rouger *et al.*, 2017). Additionally, the presence of *E. coli* in chicken meat may result from cross-contamination due to improper handling, contaminated environments, and unsanitary equipment at retail markets (Chang *et al.*, 2013). Fresh markets are typically located in open-air environments that are exposed to higher ambient temperatures and humidity levels. These conditions may lead to temperature abuse and create an environment conducive to bacterial growth (Goh *et al.*, 2025). The high rate of *E. coli* contamination in chicken meat found in the present study underscores inadequate hygiene

and food safety practices throughout the chicken meat production and distribution chain in the study area.

E. coli contamination in chicken meat was not significantly different among six different geographical regions of Bangkok in this study. Similarly, García-Béjar *et al.* (2021) reported that *E. coli* prevalence in poultry meat was not significantly influenced by geographical region. This finding may suggest that hygiene and food safety practices throughout the chicken meat production and distribution chain are relatively consistent across different regions of Bangkok, resulting in comparable levels of contamination. In addition, chicken meat sold in fresh markets across Bangkok may originate from similar suppliers or distribution networks. However, the present study did not investigate the sources of meat or the associated processing, transportation, storage, and handling practices within each region.

A major finding of this study was the high prevalence of MDR *E. coli* isolates (79.0%) recovered from chicken meat samples, highlighting a potential food safety risk associated with chicken meat consumption. High levels of MDR *E. coli* contamination in chickens and chicken meat have also been reported in other countries (Rahman *et al.*, 2020; Mgaya *et al.*, 2021). The inappropriate and excessive use of antimicrobials in food-producing animals is

considered one of the important contributing factors to the emergence and dissemination of MDR bacteria (Matheou *et al.*, 2025). In addition, poor hygiene and inadequate management practices at the farm, slaughterhouse, and retail market levels may contribute to the persistence and spread of MDR strains (Innes *et al.*, 2021).

In this study, 4.5% of *E. coli* isolates were resistant to colistin, one of the last-resort antibiotics. Alarmingly, all colistin-resistant *E. coli* isolates were classified as MDR, and 88.9% (8/9) of colistin-resistant isolates exhibited resistance to at least five antibiotics. The rate of colistin resistance observed in the present study is slightly higher than the Thailand One Health Report 2021 (HPSR-AMR, 2024), which reported a 2.8% prevalence of colistin resistance among *E. coli* isolates recovered from chicken caeca. The prevalence observed in this study is slightly lower than that reported by Sudatip *et al.* (2022) and Tangkoskul *et al.* (2021), who found colistin resistance rates of 9.1% and 7.0%, respectively, among *E. coli* isolates recovered from fecal samples from chickens and chicken offal in Thailand. The presence of colistin-resistant bacteria is especially concerning, as colistin is regarded as a last-resort antibiotic for managing infections caused by MDR Gram-negative pathogens. Infections involving colistin-resistant strains are linked to limited treatment options and elevated mortality rates (Duc *et al.*, 2024).

Only 2.0% of *E. coli* isolates in this study were confirmed as ESBL producers. Consistent with our findings, Laopiem *et al.* (2025) also reported a prevalence of 1.8% for ESBL-producing *E. coli* among isolates obtained from broiler chickens in Thailand. Conversely, the prevalence of ESBL producers in this study is substantially lower than that reported in other studies from Thailand. For instance, Dokuta *et al.* (2025) and Sornsenee *et al.* (2022) reported a prevalence of 69.9% and 79.1%, respectively, in chicken meat samples. These discrepancies could be due to variations in sampling techniques, detection methods, and antibiotic usage practices at poultry farms (Ahmed *et al.*, 2025). Notably, studies (Sornsenee *et al.*, 2022; Dokuta *et al.*, 2025) reporting high prevalence rates used ESBL-selective agar, typically supplemented with cefotaxime, to isolate *E. coli*. This selective medium enhances the growth of ESBL-producing strains, thereby increasing detection sensitivity. Such methodological differences may partly explain the lower prevalence observed in the current research compared with previous studies reporting high prevalence rates. This is further supported by Badi *et al.* (2018), who noted that the presence of ESBL-producing *E. coli* may be underestimated when non-selective protocols are used, particularly for the isolation of commensal strains.

All *E. coli* isolates in this study exhibited MAR indices ranging from 0 to 0.769. This finding is comparable to reports from India and Ghana, where the MAR indices of *E. coli* isolates from chicken meat and meat products ranged from 0 to 0.89 and 0 to 0.70, respectively (Adzitey *et al.*, 2021; Mathew *et al.*, 2025). MAR index is widely used as a simple and cost-effective indicator for assessing the potential health

risks associated with antimicrobial-resistant bacteria (Woh *et al.*, 2023). A MAR index value ≥ 0.2 suggests that bacterial isolates originate from high-risk environments with frequent antimicrobial exposure, reflecting strong selective pressures in intensive production systems and possible cross-contamination during processing (Krumperman, 1983). In the present study, 79.5% of *E. coli* isolates exhibited MAR indices greater than 0.2, indicating substantial antimicrobial selection pressure in the poultry production and retail chain. Similar findings have been reported in Malaysia, where 85.19% of Enterobacteriaceae isolates recovered from poultry and poultry meat products showed MAR index values > 0.2 (Karim *et al.*, 2023).

Ampicillin resistance was the most prevalent resistance phenotype among *E. coli* isolates in this study. To determine the factors linked to ampicillin resistance, this study performed a logistic regression analysis. Tetracycline resistance and multidrug-resistant (MDR) status were found to be statistically significant predictors. This finding aligns with a previous report by Hinthong *et al.* (2024), which demonstrated a significant association between ampicillin and tetracycline resistance in *E. coli* isolates recovered from Nile tilapia. May *et al.* (2009) also reported that the tetracycline resistance-associated gene tetA[©] and the ampicillin resistance gene blaTEM-1 are frequently encoded on mobile genetic elements, such as plasmids, transposons, and integrons, thereby supporting the potential for co-resistance mechanisms. Furthermore, Altayb *et al.* (2022) highlighted that the co-occurrence of β -lactam and aminoglycoside resistance determinants on mobile genetic elements (MGEs) facilitates the rapid development and dissemination of MDR bacteria. The strong association among ampicillin and tetracycline resistance, along with MDR status, likely reflects co-selection pressure arising from the frequent use of these antibiotics in livestock production systems (Alam *et al.*, 2023).

Among the selected AMR genes detected, the blaTEM-1 gene was the most frequently identified in *E. coli* isolates in this study. The blaTEM-1 variant encodes a narrow-spectrum β -lactamase that is commonly associated with resistance to penicillin-based antibiotics (Ahmed *et al.*, 2025). This is supported by phenotypic resistance to ampicillin in all *E. coli* isolates harboring the blaTEM-1 gene in our study. blaTEM-1 is one of the most widespread β -lactamase genes globally and is plasmid-mediated, playing a key role in spreading resistance via horizontal gene transfer (Farooq *et al.*, 2025).

Three out of four ESBL-producing *E. coli* isolates in the current study carried the blaCTX-M β -lactamase-encoding gene, while the remaining isolate tested negative for this gene. Therefore, blaCTX-M may represent a major β -lactamase gene associated with ESBL production among *E. coli* isolates in this study. Supporting this finding, Sary *et al.* (2019) hypothesized that the presence of the blaCTX-M gene was strongly associated with the dissemination of ESBL-producing *E. coli*. The blaCTX-M-14 and blaCTX-M-55 were the two blaCTX-M subtypes identified in this study. These variants have been widely reported among *E. coli*

isolates from livestock animals across Asia, particularly in Thailand, Japan, Korea, and China (Hayashi et al., 2018; Tansawai et al., 2019; Liu et al., 2020b; Kim et al., 2021; Leangapichart et al., 2023).

Among the four ESBL-producing *E. coli* strains in this study, two belonged to ST162. ST162 is a globally distributed MDR lineage that has been associated with isolates from humans, the environment, companion and livestock animals (Kawamura et al., 2017; Kindle et al., 2019; Richter et al., 2024; Slettemeås et al., 2024; Strasheim et al., 2024). Moreover, ST162 has frequently been associated with the dissemination of major ESBL genes (Hayashi et al., 2018; Isler et al., 2021).

In the present study, two colistin-resistant *E. coli* isolates and two ESBL producers were assigned to ST10, ST48, and ST209. These sequence types belong to clonal complex 10 (CC10), which is the third most common high-risk international clone (Silva et al., 2023). CC10 has been widely implicated in the dissemination of antimicrobial-resistant and pathogenic strains among humans, animals, and environmental sources worldwide (Do et al., 2022; Islam et al., 2023; Fan et al., 2024).

Interestingly, one colistin-resistant isolate in this study belonged to ST761, a high-risk lineage that has frequently been associated with the novel plasmid-mediated tigecycline resistance gene tet(X4) in China (Wang et al., 2022). Tigecycline is one of the last-resort antibiotics for application in serious infections caused by MDR, particularly carbapenem-resistant Enterobacteriaceae. However, phenotypic resistance to tigecycline and the tet(X4) gene were not investigated in this study.

This study has some limitations that should be acknowledged. First, antimicrobial usage practices in poultry production, as well as hygienic and management conditions at the farm, slaughterhouse, and retail market levels, were not evaluated. Consequently, the potential factors associated with the high occurrence of MDR *E. coli* contamination observed in this study could not be directly determined. Second, gene-specific positive controls were unavailable for some PCR assays, which may have affected the ability to fully assess assay sensitivity and specificity. To partially address this limitation, selected positive PCR amplicons were subjected to nucleotide sequencing for confirmation of target gene identity. In addition, selective media specifically designed for the isolation of ESBL-producing *E. coli* were not used, which may have resulted in underestimation of the true prevalence of ESBL-producing isolates. Future studies should incorporate detailed investigations of poultry supply chains, antimicrobial usage practices, hygienic management systems, and environmental contamination sources throughout the production and distribution chain. The use of selective isolation methods, comprehensive molecular characterization, whole-genome sequencing, and inclusion of appropriate gene-specific positive controls is also recommended to improve the detection accuracy and epidemiological understanding of antimicrobial-resistant *E. coli* in retail chicken meat. Despite these limitations, this study provides

important updated surveillance data regarding MDR, ESBL-producing, and colistin-resistant *E. coli* in retail chicken meat obtained from fresh markets in Bangkok, Thailand.

In conclusion, this study demonstrated a high prevalence of antimicrobial-resistant *E. coli* in retail chicken meat from fresh markets in Bangkok, Thailand, with a substantial proportion of isolates exhibiting MDR, ESBL production, and colistin resistance. Moreover, internationally recognized high-risk clones, such as CC10 and ST162, were found among the ESBL-producing and colistin-resistant strains. These results suggest that retail chicken meat may act as a reservoir of antimicrobial-resistant bacteria and represent a potential route for their transmission to humans through the food chain. Continuous AMR surveillance, improved hygiene practices across the poultry production and retail chain, and strengthened regulations on antimicrobial use in poultry production are essential to mitigate the spread of antimicrobial-resistant bacteria.

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Declaration of artificial intelligence use: During the preparation of this manuscript, the authors used ChatGPT (GPT-5.2; OpenAI, available at chat.openai.com) for language polishing and grammatical correction. After using this tool, the authors reviewed and edited the content as necessary and take full responsibility for the content of this publication.

References

- Adzitey F, Huda N and Shariff AHM 2021. Phenotypic antimicrobial susceptibility of *Escherichia coli* from raw meats, ready-to-eat meats, and their related samples in one health context. *Microorganisms* 9(2): 326.
- Ahmed HA, El-Tahlawy AS, El Bayomi RM, Ahmed MA, Abd Elazeem MA, Alahmad W and Hafez AE-SE 2025. Prevalence, antimicrobial resistance, and genetic profile of *Escherichia coli* in retail chicken

- parts in Zagazig City, Egypt. *Int J Food Microbiol.* 436: 111211.
- Aklilu E, Harun A and Singh KKB 2022. Molecular characterization of blaNDM, blaOXA-48, mcr-1 and blaTEM-52 positive and concurrently carbapenem and colistin resistant and extended spectrum beta-lactamase producing *Escherichia coli* in chicken in Malaysia. *BMC Vet Res.* 18(1): 190.
- Al-Mazrouei MA, Al-Kharousi ZS, Al-Kharousi JM and Al-Barashdi HM 2024. Microbiological evaluation of local and imported raw beef meat at retail sites in Oman with emphasis on spoilage and pathogenic psychrotrophic bacteria. *Microorganisms* 12(12): 2545.
- Al Azad MAR, Rahman MM, Amin R, Begum MIA, Fries R, Husna A, Khairalla AS, Badruzzaman A, El Zowalaty ME and Lampang KN 2019. Susceptibility and multidrug resistance patterns of *Escherichia coli* isolated from cloacal swabs of live broiler chickens in Bangladesh. *Pathogens* 8(3): 118.
- Alam GS, Hassan MM, Ahaduzzaman M, Nath C, Dutta P, Khanom H, Khan SA, Pasha MR, Islam A and Magalhaes RS 2023. Molecular detection of tetracycline-resistant genes in multi-drug-resistant *Escherichia coli* isolated from broiler meat in Bangladesh. *Antibiotics* 12(2): 418.
- Altayb HN, Elbadawi HS, Alzahrani FA, Baothman O, Kazmi I, Nadeem MS, Hosawi S and Chaieb K 2022. Co-occurrence of β -lactam and aminoglycoside resistance determinants among clinical and environmental isolates of *Klebsiella pneumoniae* and *Escherichia coli*: A genomic approach. *Pharmaceuticals* 15(8): 1011.
- Ananchaipattana C, Hosotani Y, Kawasaki S, Pongsawat S, Md. Latiful B, Isobe S and Inatsu Y 2012. Prevalence of foodborne pathogens in retailed foods in Thailand. *Foodborne Pathog Dis.* 9(9): 835–840.
- Badi S, Cremonesi P, Abbassi MS, Ibrahim C, Snoussi M, Bignoli G, Luini M, Castiglioni B and Hassen A 2018. Antibiotic resistance phenotypes and virulence-associated genes in *Escherichia coli* isolated from animals and animal food products in Tunisia. *FEMS Microbiol Lett.* 365(10): fny088.
- Batchelor M, Hopkins K, Threlfall E, Clifton-Hadley F, Stallwood A, Davies R and Liebana E 2005. blaCTX-M genes in clinical *Salmonella* isolates recovered from humans in England and Wales from 1992 to 2003. *Antimicrob Agents Chemother.* 49(4): 1319–1322.
- Borowiak M, Baumann B, Fischer J, Thomas K, Deneke C, Hammerl JA, Szabo I and Malorny B 2020. Development of a novel mcr-6 to mcr-9 multiplex PCR and assessment of mcr-1 to mcr-9 occurrence in colistin-resistant *Salmonella enterica* isolates from environment, feed, animals and food (2011–2018) in Germany. *Front Microbiol.* 11: 80.
- Chaisatit C, Tribuddharat C, Pulsrikarn C and Dejsirilert S 2012. Molecular characterization of antibiotic-resistant bacteria in contaminated chicken meat sold at supermarkets in Bangkok, Thailand. *Jpn J Infect Dis.* 65(6): 527–534.
- Chang W, Afsah-Hejri L, Rukayadi Y, Khatib A, Lye Y, Loo Y, Shahril NM, Puspanadan S, Kuan C and Goh S 2013. Quantification of *Escherichia coli* O157:H7 in organic vegetables and chickens. *Int Food Res J.* 20(2): 1023–1029.
- Chotinantakul K, Chusri P and Okada S 2022. Detection and characterization of ESBL-producing *Escherichia coli* and additional co-existence with mcr genes from river water in northern Thailand. *PeerJ* 10: e14408.
- CLSI 2024a. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. 6th ed. Wayne, PA: Clinical and Laboratory Standards Institute. 164 pp.
- Missing reference for CLSI 2024b
- Department of Trade Negotiation T 2021. “Summary report on the production and export of chicken meat and products 2020.” [Online]. Available: <https://api.dtn.go.th/files/v3/60f91710ef4140b37906fdcf/download>. Accessed February 15, 2026.
- Do K-H, Seo K and Lee W-K 2022. Antimicrobial resistance, virulence genes, and phylogenetic characteristics of pathogenic *Escherichia coli* isolated from patients and swine suffering from diarrhea. *BMC Microbiol.* 22(1): 199.
- Dokuta S, Yadoung S, Hongjaisee S, Khamnoi P, Manochomphu S, Chuttong B and Hongsisong S 2025. Seasonal determination of antibiotic-resistant microorganisms and ciprofloxacin residues in pork and chicken meats collected from fresh markets in Chiang Mai, northern Thailand. *Foods* 14(2): 174.
- Duc HM, Hoa TTK, Thang NV and Son HM 2024. First report on the occurrence and antibiotic resistance profile of colistin-resistant *Escherichia coli* in raw beef and cow feces in Vietnam. *Microorganisms* 12(7): 1305.
- Edelman DJ 2022. Managing the urban environment of Bangkok, Thailand. *Curr Urban Stud.* 10(1): 73–92.
- Elmonir W, Abd El-Aziz NK, Tartor YH, Moustafa SM, Abo Remela EM, Eissa R, Saad HA and Tawab AA 2021. Emergence of colistin and carbapenem resistance in extended-spectrum β -lactamase producing *Klebsiella pneumoniae* isolated from chickens and humans in Egypt. *Biology* 10(5): 373.
- Fan X-Y, Jiang Y, Wu H, Liu J, Gu Q-Y, Wang Z-Y, Sun L, Jiao X, Li Q and Wang J 2024. Distribution and spread of tigecycline resistance gene tet (X4) in *Escherichia coli* from different sources. *Front Cell Infect Microbiol.* 14: 1399732.
- Farooq A, Rafique A, Han E and Park S-M 2025. Global dissemination of the beta-lactam resistance gene blaTEM-1 among pathogenic bacteria. *Sci Total Environ.* 963: 178521.
- Feng P, Weagant SD, Grant MA and Burkhardt W 2020. Enumeration of *Escherichia coli* and the coliform bacteria. In: *Bacteriological Analytical Manual (BAM)*. 8th ed. Washington DC: U.S. Food and Drug Administration. 2–18.
- García-Béjar B, García de Blas Martín I, Arévalo-Villena M and Briones Pérez A 2021. High prevalence of antibiotic-resistant *Escherichia coli* isolates from retail poultry products in Spain. *Animals* 11(11): 3197.

- Goh SG, Radu S, Haryani Y, Epeng L, Nor-Khaizura MAR, Abdul-Mutalib NA and Hasan H 2025. High prevalence of antibiotic-resistant *Escherichia coli* harbouring extended-spectrum beta-lactamase (ESBL) in raw chicken meat sold at retail in Klang Valley, Malaysia. FEMS Microbiol Lett. 372: fnaf039.
- Göpel L, Prenger-Berninghoff E, Wolf SA, Semmler T, Bauerfeind R and Ewers C 2023. Occurrence of mobile colistin resistance genes mcr-1-mcr-10 including novel mcr gene variants in different pathotypes of porcine *Escherichia coli* isolates collected in Germany from 2000 to 2021. Appl Microbiol. 4(1): 70–84.
- Hall TA 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 41: 95–98.
- Hasman H, Mevius D, Veldman K, Olesen I and Aarestrup FM 2005. β -Lactamases among extended-spectrum β -lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. J Antimicrob Chemother. 56(1): 115–121.
- Hayashi W, Ohsaki Y, Taniguchi Y, Koide S, Kawamura K, Suzuki M, Kimura K, Wachino J-i, Nagano Y and Arakawa Y 2018. High prevalence of blaCTX-M-14 among genetically diverse *Escherichia coli* recovered from retail raw chicken meat portions in Japan. Int J Food Microbiol. 284: 98–104.
- Hinthong W, Thaotumpitak V, Sripradite J, Indrawattana N, Srisook T, Kongngoen T, Atwill ER and Jeamsripong S 2024. Antimicrobial resistance, virulence profile, and genetic analysis of esbl-producing *Escherichia coli* isolated from Nile tilapia in fresh markets and supermarkets in Thailand. PLoS One 19(1): e0296857.
- HPSR-AMR 2024. "Health policy and systems research on antimicrobial resistance (HPSR-AMR) network. Highlights Thailand one health report 2021: Antimicrobial consumption and antimicrobial resistance." [Online]. Available: <https://www.thaiamrwatch.net/materials/>. Accessed February 15, 2026.
- Innes GK, Nachman KE, Abraham AG, Casey JA, Patton AN, Price LB, Tartof SY and Davis MF 2021. Contamination of retail meat samples with multidrug-resistant organisms in relation to organic and conventional production and processing: A cross-sectional analysis of data from the United States National Antimicrobial Resistance Monitoring System, 2012–2017. Environ Health Perspect. 129(5): 057004.
- Islam MS, Rahman AT, Hassan J and Rahman MT 2023. Extended-spectrum beta-lactamase in *Escherichia coli* isolated from humans, animals, and environments in Bangladesh: A one health perspective systematic review and meta-analysis. One Health. 16: 100526.
- Isler M, Wissmann R, Morach M, Zurfluh K, Stephan R and Nüesch-Inderbinen M 2021. Animal petting zoos as sources of shiga toxin-producing *Escherichia coli*, *Salmonella* and extended-spectrum β -lactamase (esbl)-producing Enterobacteriaceae. Zoonoses Public Health. 68(2): 79–87.
- Karim MR, Zakaria Z, Hassan L, Mohd Faiz N and Ahmad NI 2023. Antimicrobial resistance profiles and co-existence of multiple antimicrobial resistance genes in mcr-harboring colistin-resistant Enterobacteriaceae isolates recovered from poultry and poultry meats in Malaysia. Antibiotics 12(6): 1060.
- Kawamura K, Sugawara T, Matsuo N, Hayashi K, Norizuki C, Tamai K, Kondo T and Arakawa Y 2017. Spread of ctx-type extended-spectrum β -lactamase-producing *Escherichia coli* isolates of epidemic clone b2-o25-st131 among dogs and cats in Japan. Microb Drug Resist. 23(8): 1059–1066.
- Kim H, Kim YA, Seo YH, Lee H and Lee K 2021. Prevalence and molecular epidemiology of extended-spectrum- β -lactamase (esbl)-producing *Escherichia coli* from multiple sectors of poultry industry in Korea. Antibiotics 10(9): 1050.
- Kim S, Kim H, Kim Y, Kim M, Kwak H and Ryu S 2020. Antimicrobial resistance of *Escherichia coli* from retail poultry meats in Korea. J Food Prot. 83(10): 1673–1678.
- Kindle P, Zurfluh K, Nüesch-Inderbinen M, von Ah S, Sidler X, Stephan R and Kümmerlen D 2019. Phenotypic and genotypic characteristics of *Escherichia coli* with non-susceptibility to quinolones isolated from environmental samples on pig farms. Porcine Health Manag. 5: 1–12.
- Krumperman PH 1983. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. Appl Environ Microbiol. 46(1): 165–170.
- Laopiem S, Witoonsatien K, Kulprasetsri S, Panomwan P, Pathomchai-Umporn C, Kamtae R, Jirawattanapong P, Songserm T and Sinwat N 2025. Antimicrobial resistance, virulence gene profiles, and phylogenetic groups of *Escherichia coli* isolated from healthy broilers and broilers with colibacillosis in Thailand. BMC Vet Res. 21(1): 160.
- Lay KK, Jeamsripong S, Sunn KP, Angkititrakul S, Prathan R, Srisanga S and Chuanchuen R 2021. Colistin resistance and esbl production in salmonella and *escherichia coli* from pigs and pork in the Thailand, Cambodia, Lao PDR, and Myanmar border area. Antibiotics 10(6): 657.
- Leangapichart T, Stosic MS, Hickman RA, Lunha K, Jiwakanon J, Angkititrakul S, Magnusson U, Van Boeckel TP, Järhult JD and Sunde M 2023. Exploring the epidemiology of mcr genes, genetic context and plasmids in Enterobacteriaceae originating from pigs and humans on farms in Thailand. J Antimicrob Chemother. 78(6): 1395–1405.
- Levesque C, Piche L, Larose C and Roy PH 1995. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob Agents Chemother. 39(1): 185–191.
- Mgaya FX, Matee MI, Muhairwa AP and Hoza AS 2021. Occurrence of multidrug resistant *Escherichia coli* in raw meat and cloaca swabs in poultry

- processed in slaughter slabs in Dar es Salaam, Tanzania. *Antibiotics* 10(4): 343.
- Moawad AA, Hotzel H, Neubauer H, Ehrlich R, Monecke S, Tomaso H, Hafez HM, Roesler U and El-Adawy H 2018. Antimicrobial resistance in Enterobacteriaceae from healthy broilers in Egypt: Emergence of colistin-resistant and extended-spectrum β -lactamase-producing *Escherichia coli*. *Gut Pathog.* 10: 1–12.
- Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, Han C, Bisignano C, Rao P and Wool E 2022. Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *Lancet* 399(10325): 629–655.
- Muzaheed, Doi Y, Adams-Haduch JM, Endimiani A, Sidjabat HE, Gaddad SM and Paterson DL 2008. High prevalence of CTX-M-15-producing *Klebsiella pneumoniae* among inpatients and outpatients with urinary tract infection in Southern India. *J Antimicrob Chemother.* 61(6): 1393–1394.
- NARMS 2024. “National antimicrobial resistance monitoring system; antibiotics used for susceptibility testing of *E. coli* isolates.” [Online]. Available: <https://www.cdc.gov/narms/about/antibiotics-tested.html>. Accessed May 17, 2025.
- NCBI 2024. “National center for biotechnology information (NCBI). GenBank and BLAST tools.” [Online]. Available: <https://www.ncbi.nlm.nih.gov/>. Accessed April 19, 2025.
- O'Neill J 2016. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. The Review on Antimicrobial Resistance. 84 pp.
- Olesen I, Hasman H and Møller Aarestrup F 2004. Prevalence of β -lactamases among ampicillin-resistant *Escherichia coli* and *Salmonella* isolated from food animals in Denmark. *Microb Drug Resist.* 10(4): 334–340.
- Pires J, Huisman JS, Bonhoeffer S, and Van Boeckel TP 2022. Increase in antimicrobial resistance in *Escherichia coli* in food animals between 1980 and 2018 assessed using genomes from public databases. *J Antimicrob Chemother.* 77(3): 646–655.
- Poirel L, Madec J-Y, Lupo A, Schink A-K, Kieffer N, Nordmann P and Schwarz S 2018. Antimicrobial resistance in *Escherichia coli*. *Microbiol Spectr.* 6(4): 10.1128/microbiolspec.arba-0026-2017.
- Poirel L, Walsh TR, Cuvillier V and Nordmann P 2011. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis.* 70(1): 119–123.
- Pumart P, Phodha T, Thamlikitkul V, Riewpaiboon A, Prakongsai P and Limwattananon S 2012. Health and economic impacts of antimicrobial resistance in Thailand. *J Health Serv Res Policy.* 6(3): 352–360.
- Puvača N and de Llanos Frutos R 2021. Antimicrobial resistance in *Escherichia coli* strains isolated from humans and pet animals. *Antibiotics* 10(1): 69.
- Rahman MM, Husna A, Elshabrawy HA, Alam J, Runa NY, Badruzzaman A, Banu NA, Al Mamun M, Paul B and Das S 2020. Isolation and molecular characterization of multidrug-resistant *Escherichia coli* from chicken meat. *Sci Rep.* 10(1): 21999.
- Richter L, Duvenage S, du Plessis EM, Msimango T, Dlangalala M, Mathavha MT, Molelekoa T, Kgoale DM and Korsten L 2024. Genomic evaluation of multidrug-resistant extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* from irrigation water and fresh produce in South Africa: A cross-sectional analysis. *Environ Sci Technol.* 58(32): 14421–14438.
- Rouger A, Tresse O and Zagorec M 2017. Bacterial contaminants of poultry meat: Sources, species, and dynamics. *Microorganisms* 5(3): 50.
- Runcharoen C, Raven KE, Reuter S, Kallonen T, Paksanont S, Thammachote J, Anun S, Blane B, Parkhill J and Peacock SJ 2017. Whole genome sequencing of ESBL-producing *Escherichia coli* isolated from patients, farm waste and canals in Thailand. *Genome Med.* 9(1): 81.
- Sabaté M, Tarragó RI, Navarro F, Miró E, Vergés C, Barbé J and Prats G 2000. Cloning and sequence of the gene encoding a novel cefotaxime-hydrolyzing β -lactamase (CTX-M-9) from *Escherichia coli* in Spain. *Antimicrob Agents Chemother.* 44(7): 1970–1973.
- Sary K, Fairbrother JM, Arsenault J, de Lagarde M and Boulianne M 2019. Antimicrobial resistance and virulence gene profiles among *Escherichia coli* isolates from retail chicken carcasses in Vietnam. *Foodborne Pathog Dis.* 16(4): 298–306.
- Silva A, Silva V, Pereira JE, Maltez L, Igrejas G, Valentão P, Falco V and Poeta P 2023. Antimicrobial resistance and clonal lineages of *Escherichia coli* from food-producing animals. *Antibiotics* 12(6): 1061.
- Slette-meås JS, Sekse C, Sunde M, Norström M, Wester AL, Naseer U, Simonsen GS, Ulstad CR, Urdahl AM and Lagesen K 2024. Comparative genomics of quinolone-resistant *Escherichia coli* from broilers and humans in Norway. *BMC Microbiol.* 24(1): 248.
- Sornsenee P, Chimplee S, Arububaker A, Kongchai S, Madimong H and Romyasamit C 2022. Occurrence, antimicrobial resistance profile, and characterization of extended-spectrum β -lactamase-producing *Escherichia coli* isolates from minced meat at local markets in Thailand. *Foodborne Pathog Dis.* 19(3): 232–240.
- Strasheim W, Lowe M, Smith AM, Etter EM and Perovic O 2024. Whole-genome sequencing of human and porcine *Escherichia coli* isolates on a commercial pig farm in South Africa. *Antibiotics* 13(6): 543.
- Sudatip D, Tiengrim S, Chasiri K, Kritiyakan A, Phanprasit W, Morand S and Thamlikitkul V 2022. One health surveillance of antimicrobial resistance phenotypes in selected communities in Thailand. *Antibiotics* 11(5): 556.
- Tangkoskul T, Thamthaweechok N, Seenama C and Thamlikitkul V 2021. Antibiotic-resistant bacteria and antibiotic residue contamination in fresh raw foods sold at wholesale markets in Thailand. *J Med Assoc Thai.* 104: 654–662.

- Tansawai U, Walsh TR and Niumsup PR 2019. Extended spectrum β -lactamase-producing *Escherichia coli* among backyard poultry farms, farmers, and environments in Thailand. Poult Sci. 98(6): 2622–2631.
- Tartof SY, Solberg OD, Manges AR and Riley LW 2005. Analysis of a uropathogenic *Escherichia coli* clonal group by multilocus sequence typing. J Clin Microbiol. 43(12): 5860–5864.
- Thrusfield M 2018. Veterinary Epidemiology. 4th ed. Hoboken: John Wiley & Sons. 864 pp.
- Trongjit S, Angkittitrakul S and Chuanchuen R 2016. Occurrence and molecular characteristics of antimicrobial resistance of *Escherichia coli* from broilers, pigs and meat products in Thailand and Cambodia provinces. Microbiol Immunol. 60(9): 575–585.
- Wang J, Lu M-J, Wang Z-Y, Jiang Y, Wu H, Pan Z-M and Jiao X 2022. Tigecycline-resistant *Escherichia coli* ST761 carrying tet (X4) in a pig farm, China. Front Microbiol. 13: 967313.
- WHO 2023. “Antimicrobial resistance.” [Online]. Available: <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance>. Accessed February 19, 2026.
- Woh PY, Yeung MPS and Goggins III WB 2023. Multiple antibiotic resistance index (MARI) of human-isolated *Salmonella* species: A practical bacterial antibiotic surveillance tool. J Antimicrob Chemother. 78(5): 1295–1299.
- Zellweger RM, Carrique-Mas J, Limmathurotsakul D, Day NPJ, Thwaites GE, Baker S and Network SAAR 2017. A current perspective on antimicrobial resistance in Southeast Asia. J Antimicrob Chemother. 72(11): 2963–2972.