



Prevalence and Antimicrobial Resistance of Multidrug-Resistant *Salmonella* spp. in Retail Chicken Outlets in Phatthalung, Thailand

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Abstract: Salmonellosis is a major foodborne disease associated with the consumption of contaminated poultry products. This study aimed to determine the prevalence and antimicrobial resistance of *Salmonella* spp. isolated from retail chicken outlets in Phatthalung, Thailand. A total of 43 isolates recovered from chicken meat, knives, and cutting boards across five outlets were confirmed via biochemical tests and PCR using genus-specific primers. Serotyping revealed six serogroups, with serogroup B being the most prevalent (37.21%), followed by C2 (18.60%), C1 (16.28%), D and E (11.63% each), and G (4.65%). Antimicrobial susceptibility testing against 13 antibiotics showed high resistance to ampicillin (95.35%), streptomycin (69.77%), and tetracycline (60.46%). Moderate resistance to fluoroquinolones was detected (41.86–46.51%), whereas gentamicin showed comparatively lower resistance (23.26%). In contrast, resistance to third-generation cephalosporins remained low (ceftriaxone 4.65%; cefotaxime 13.95%). Identical antibiogram patterns between meat samples and environmental surfaces (knives and cutting boards) within the same outlets suggested possible cross-contamination. Notably, 30 (69.77%) isolates were multidrug-resistant *Salmonella*, resistant to 3 or more antibiotic classes. Therefore, these findings highlight the role of retail chicken outlets as reservoirs of antimicrobial-resistant *Salmonella*, posing a potential public health risk.

Keywords: *Salmonella*; retail chicken outlet; antimicrobial resistance; prevalence

1. Introduction

Salmonella is a genus of Gram-negative, rod-shaped, facultatively anaerobic bacteria belonging to the family *Enterobacteriaceae*, and is a major cause of foodborne infection in humans and animals worldwide. Within this genus, only two species are classified: *S. enterica* and *S. bongori*. *S. enterica* is further divided into six subspecies: *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), and *indica* (VI) [1]. Notably, *S. enterica* subsp. *enterica* is the most clinically significant [2]. Serotyping is the conventional method widely used for the classification and surveillance of *Salmonella* in clinical and veterinary settings. Based on the White–Kauffmann–Le Minor scheme, *S. enterica* is classified into more than 60 serogroups and 2,600 serotypes, determined by unique combinations of O and H antigens [3]. Based on pathogenicity and host range, *S. enterica* serovars are classified into two groups: typhoidal and non-typhoidal. Typhoidal serovars, such as *S. Typhi*

and *S. Paratyphi*, are human-restricted pathogens that cause typhoid fever, a systemic and potentially life-threatening disease [4]. In contrast, non-typhoidal serovars (NTS), including *S. Typhimurium* and *S. Enteritidis*, infect a wide range of hosts and are typically associated with self-limiting gastroenteritis, although invasive disease may occur [3]. NTS is among the most common causes of foodborne illness worldwide. In Thailand, NTS infection remains an important public health concern. A nationwide study conducted in 2022 reported approximately 4,501 cases and 461 deaths associated with NTS infection [5].

Poultry is recognized as a major reservoir of *Salmonella*. The organism is commonly present in the gastrointestinal tract of poultry and often contaminates carcasses during slaughter or processing. Consequently, *Salmonella* can be transmitted to humans through direct contact with poultry or indirectly through the consumption of contaminated chicken meat and cross-contaminated products [6]. In addition, the emergence of antimicrobial resistance (AMR) in *Salmonella* spp., particularly multidrug-resistant (MDR) strains, poses a serious global public health threat [7]. This issue is largely driven by the extensive use of antimicrobials in human medicine, agriculture, and livestock production [8]. Several studies have reported that *Salmonella* isolated from poultry meat frequently exhibit resistance to multiple antimicrobial agents [9-13]. Infections caused by MDR *Salmonella* may result in more severe clinical outcomes and prolonged hospitalization [14].

In Southern Thailand, where chicken meat is widely consumed and wet-market retail trade is the dominant food distribution model, monitoring the prevalence and antimicrobial resistance of *Salmonella* in poultry is essential to ensure food safety. Phatthalung Province, in particular, supports a substantial small-scale poultry industry supplying local wet markets, yet systematic data on the AMR burden of *Salmonella* at the retail level in this province are lacking. Studies in the broader Southern Thailand region have documented antimicrobial residues in retail pork and chicken [15], MDR *Escherichia coli* O45 in raw meats [16], methicillin-resistant *Staphylococcus aureus* in retail meats [17], and microbial contamination in locally processed food products in Phatthalung [18, 19], highlighting the need for systematic surveillance of foodborne pathogens in this region. This study, therefore, aimed to determine the prevalence, serogroup distribution, and antimicrobial resistance patterns of *Salmonella* spp. isolated from chicken meat and environmental surfaces (knives and cutting boards) in retail chicken outlets in Phatthalung, Thailand. The findings of this study provide baseline data for understanding the role of retail environments in the dissemination of antimicrobial-resistant *Salmonella* and may support the development of strategies to reduce the risk of foodborne transmission. Specifically, the data generated will inform targeted antibiotic stewardship programs and hygiene-intervention policies for wet-market retail settings in Southern Thailand.

2. Materials and Methods

2.1 Sample collection

Samples were collected from five retail chicken outlets located in wet markets from three districts of Phatthalung, Thailand, between June and July 2024. Outlet 1 was located in Pa Payom, outlets 2 and 5 were located in Kuan Kanoon, and outlets 3 and 4 were located in Mueang District. From each outlet, five raw chicken meat samples, ten cutting board swab samples, and ten knife swab samples were collected. All samples were placed in separate sterile plastic bags and immediately transported to the laboratory in an ice box at 4 °C for bacterial isolation. Swab samples from cutting boards and knives were collected directly at the outlets, whereas meat samples were purchased from the vendors. For swab sample collection, sterile, pre-moistened swabs in phosphate-buffered saline (PBS) were used. Each swab was applied to approximately 6.45 cm² of the surface area.

2.2 Isolation of *Salmonella* spp.

Isolation of *Salmonella* spp. was performed according to ISO 6579:2017 with slight modifications. The modifications consisted of reducing the pre-enrichment volume for swab samples (10 mL BPW instead of 225 mL) to accommodate the smaller sample mass. For meat samples, 25 g of chopped meat was aseptically transferred into 225 mL of buffered peptone water (BPW). For knife and cutting board samples, each swab was placed into 10 mL of BPW. All samples were incubated at 37 °C for 18–24 hours for pre-enrichment. Subsequently, 0.1 mL of the pre-enrichment culture was transferred into 9.9 mL of Rappaport–Vassiliadis soya

(RVS) broth and incubated at 42 °C for 24 hours, while 1 mL was inoculated into 9 mL of Muller–Kauffmann tetrathionate novobiocin (MKTn) broth and incubated at 37 °C for 24 hours. Both RVS and MKTn broths were used simultaneously because their complementary selectivities increase overall *Salmonella* recovery: RVS is more effective for low-inoculum samples, while MKTn supports a broader range of serovars. After selective enrichment, a loopful of culture from each broth was streaked onto xylose lysine deoxycholate (XLD) agar and *Salmonella*–*Shigella* (SS) agar plates, then incubated at 37 °C for 24 hours. Presumptive *Salmonella* colonies, characterized by a transparent appearance with a black center, were selected and purified on XLD agar. Pure isolates were subsequently maintained on tryptic soy agar (TSA) slants for further identification.

2.3 Identification and Serogrouping of *Salmonella* spp.

Presumptive isolates were initially identified by Gram staining and standard biochemical tests, including motility–indole–lysine (MIL), triple sugar iron (TSI), and urease tests. Serological confirmation was performed by slide agglutination using polyvalent O antisera (A–I) (St Serotest). Isolates showing positive agglutination were further confirmed at the genus level by polymerase chain reaction (PCR) targeting the *invA* gene using primers *invA*-F (5'-CGGTGGTTTTAAGCGTACTCTT-3') and *invA*-R (5'-CGAATATGCTCCACAAGGTTA-3'), yielding a 796 bp amplicon [20]. Serogrouping was subsequently determined by slide agglutination using polyvalent antisera specific for groups A, B, C1, C2, C3, D, E, and G (St Serotest). It should be noted that definitive serotyping to the serovar level was achieved only for *S. Enteritidis* and *S. Typhimurium* by multiplex PCR; all remaining isolates were classified only to the serogroup level.

2.4 Antimicrobial susceptibility test

The antimicrobial resistance patterns of *Salmonella* isolates were determined using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar, according to the Clinical and Laboratory Standards Institute guidelines [21]. A total of 13 commercially available antibiotic disks (Oxoid) were used: ampicillin (10 µg; AMP), cefotaxime (30 µg; CTX), ceftriaxone (30 µg; CTR), nalidixic acid (30 µg; NA), enrofloxacin (5 µg; ENR), ciprofloxacin (5 µg; CIP), levofloxacin (5 µg; LEV), tetracycline (30 µg; TE), doxycycline (30 µg; DO), gentamicin (10 µg; GEN), streptomycin (10 µg; S), chloramphenicol (15 µg; C), and trimethoprim (5 µg; W). *Escherichia coli* ATCC 25922 was used as a quality control strain. The results were interpreted as susceptible, intermediate, or resistant based on the inhibition zone diameters according to CLSI criteria. Updated breakpoints from CLSI M100 (2023) were also consulted, particularly for fluoroquinolones. Isolates with intermediate susceptibility results were recorded separately and were not grouped with resistant isolates in the prevalence calculations reported herein. This culture-dependent AST approach, when combined with molecular characterization, is consistent with the methods used in comparable AMR studies [22].

2.5 Multiplex Polymerase Chain Reaction for the presence of *invA*, *IE1*, and *fliC* genes

One to two colonies from overnight cultures were transferred into 0.2 mL of sterile distilled water and heated to 100 °C for 5 minutes. The lysate was then used as the template DNA for PCR analysis. Multiplex PCR (mPCR) was performed using three sets of specific primers targeting the *invA*, *IE1*, and *fliC* genes for the detection of *Salmonella* spp., *S. Enteritidis*, and *S. Typhimurium*, respectively, as previously described by Paiao et al. (2013) [20]. Briefly, each 25 µL PCR reaction mixture consisted of 2.5 µL of 10x PCR buffer, 1 µL of dNTP mix (10 µM each), 0.5 µL of each primer (10 µM), 0.25 µL (1.25 U) of Taq DNA polymerase, 1 µL of template DNA, and nuclease-free water to a final volume of 25 µL. The mPCR amplification protocol included an initial denaturation step at 95 °C for 5 minutes, followed by 30 cycles of denaturation at 95 °C for 1 minute, annealing at 58 °C for 1 minute, and extension at 72 °C for 30 seconds, with a final extension step at 72 °C for 7 minutes. PCR products were analyzed by electrophoresis on a 1.5% agarose gel using Tris-acetate-EDTA (TAE) buffer. The expected amplicon sizes were 796 bp for *invA* (*Salmonella* spp.), 432 bp for *fliC* (*S. Typhimurium*), and 316 bp for *IE1* (*S. Enteritidis*).

3. Results

In this study, *Salmonella* was detected in all sampled retail chicken outlets, including chicken meat, knives, and cutting boards. A total of forty-three isolates ($n = 43$) were systematically identified as *Salmonella* spp. based on biochemical, serological, and molecular characterization. All isolates were Gram-negative, exhibited K/A with H₂S production in TSI, motile, negative for indole and urease, positive for lysine decarboxylase, agglutinated with polyvalent A-I antiserum, and carried the *invA* gene. These isolates were subsequently characterized by slide agglutination and tested for antimicrobial susceptibility to 13 antibiotics by the disc diffusion method. The antimicrobial agents are categorized into 7 classes: penicillins (AMP), third-generation cephalosporins (CTX and CTR), quinolones (NA, ENR, CIP, and LEV), tetracyclines (TE and DO), aminoglycosides (GEN and S), phenicols (C), and diaminopyrimidines (W). The 43 *Salmonella* isolates were classified into six serogroups (Table 1). Serogroup B was the most prevalent (37.21%, 16/43), followed by serogroup C (34.88%, 15/43), which comprised C2 (18.60%, 8/43) and C1 (16.28%, 7/43). Serogroups D and E were each detected in 5 isolates (11.63%), while serogroup G was the least prevalent (4.65%, 2/43). Among the serogroups B and D isolates, 7 (16.28%) and 2 (4.65%) isolates were molecularly identified as *S. Typhimurium* (*invA*⁺ and *fliC*⁺) and *S. Enteritidis* (*invA*⁺ and *IE1*⁺), respectively (Figure 1). Notably, all isolates of these two serovars exhibited multidrug resistance (MDR). *S. Typhimurium* showed resistance patterns 10, 13, 16, and 18, whereas *S. Enteritidis* displayed resistance patterns 11 and 16 (Table 2).

All 43 isolates (100%) exhibited resistance to at least 1 antibacterial agent. High levels of resistance were observed for several antibacterial agents, including ampicillin (95.35%), streptomycin (69.77%), tetracycline (60.46%), doxycycline (46.51%), nalidixic acid (46.51%), ciprofloxacin (41.86%), enrofloxacin (41.86%), and levofloxacin (41.86%). On the other hand, low resistance was observed with third-generation cephalosporins, including ceftriaxone (4.65%) and cefotaxime (13.95%). Furthermore, moderate levels of resistance were also observed for gentamicin (23.26%), chloramphenicol (27.91%), and trimethoprim (34.88%) (Table 1).

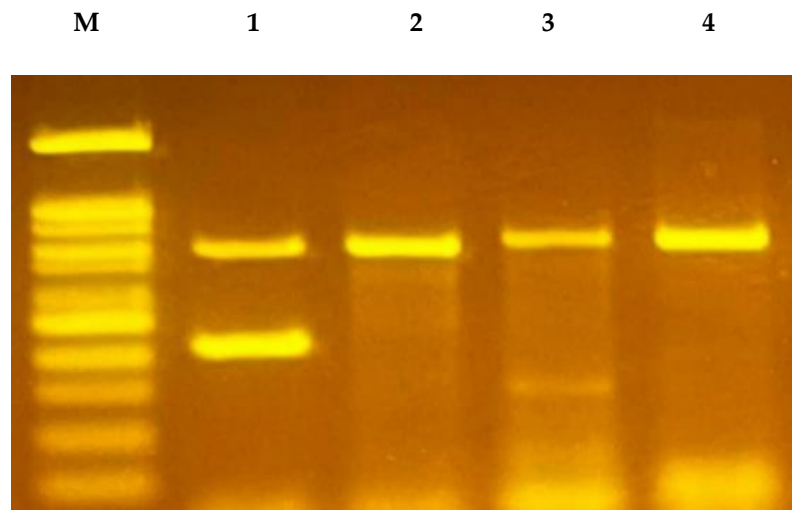


Figure 1. Multiplex PCR detection of *invA* (796 bp), *fliC* (432 bp), and *IE1* (316 bp) genes in *Salmonella* isolates. Lane M: DNA ladder; lane 1: *S. Typhimurium* (serogroup B); lane 2: *Salmonella* spp. (serogroup C1); lane 3: *S. Enteritidis* (serogroup D), and lane 4: *Salmonella* spp. (serogroup E).

Table 1. Serogroup distribution and antimicrobial resistance profiles of *Salmonella* spp. (n = 43) isolated from 5 retail chicken outlets in Phatthalung, Thailand.

Serogroup (outlet)	Number of isolates (%)	Number of resistant isolates (%)												
		AMP	CTX	CTR	NA	CIP	ENR	LEV	TE	DO	GEN	S	C	W
B	16	14	0	0	7	7	7	7	9	7	2	8	4	5
(1-5)	(37.21)	(87.50)	(0)	(0)	(43.75)	(43.75)	(43.75)	(43.75)	(56.25)	(43.75)	(12.50)	(50.00)	(25.00)	(31.25)
C1	7	7	3	2	4	3	3	3	6	4	3	7	5	5
(1-4)	(16.28)	(100)	(42.86)	(28.57)	(57.14)	(42.86)	(42.86)	(42.86)	(85.71)	(57.14)	(42.86)	(100)	(71.43)	(71.43)
C2	8	8	1	0	3	2	2	2	5	5	2	5	0	1
(1-4)	(18.60)	(100)	(12.50)	(0)	(37.50)	(25)	(25)	(25)	(62.50)	(62.50)	(25)	(62.50)	(0)	(12.50)
D	5	5	0	0	2	2	2	2	1	1	0	3	0	0
(1, 4, 5)	(11.63)	(100)	(0)	(0)	(40)	(40)	(40)	(40)	(20)	(20)	(0)	(60)	(0)	(0)
E	5	5	0	0	2	2	2	2	4	2	3	5	2	4
(1, 2, 3)	(11.63)	(100)	(0)	(0)	(40)	(40)	(40)	(40)	(80)	(40)	(60)	(100)	(40)	(80)
G	2	2	2	0	2	2	2	2	1	1	0	2	1	0
(3, 4)	(4.65)	(100)	(100)	(0)	(100)	(100)	(100)	(100)	(50)	(50)	(0)	(100)	(50)	(0)
Total	43	41	6	2	20	18	18	18	26	20	10	30	12	15
	(100)	(95.35)	(13.95)	(4.65)	(46.51)	(41.86)	(41.86)	(41.86)	(60.46)	(46.51)	(23.26)	(69.77)	(27.91)	(34.88)

This study revealed 21 distinct antimicrobial resistance patterns among *Salmonella* isolates, indicating strain diversity in this area. However, similar resistance profiles were observed among isolates in different serogroups. Resistance pattern 10 was found in both serogroups B and C1, while pattern 11 was shared across serogroups B, C1, and D. Furthermore, pattern 16 was detected in the isolates from serogroups B, C2, and D, while pattern 18 was distributed among serogroups B, C1, and E (Table 2). Thirty isolates (69.77%) were multidrug-resistant *Salmonella* spp. (pattern 7-21), defined as resistance to at least one antimicrobial agent in three or more antimicrobial classes. However, MDR profiles were investigated in all serogroups. Of note, some isolates exhibited resistance to 9-12 antibiotics (patterns 19 and 21) across seven antimicrobial classes, highlighting the presence of extensively drug-resistant (XDR) strains. The most prevalent MDR profiles were AMP-NA-ENR-CIP-LEV-TE-DO-S-W (13.95%) and AMP-NA-ENR-CIP-LEV-TE-DO-S (9.30%). The multiple antibiotic resistance (MAR) index ranged from 0.08 to 0.92. Notably, 31 isolates (72.09%) exhibited MAR values greater than 0.2, and 16 isolates (37.21%) showed index values greater than 0.5.

In 4 of 5 (80%) retail chicken outlets, similar antimicrobial resistance patterns and serogroups were investigated among *Salmonella* isolates recovered from different sample types (meat, board, and knife) within the same retail outlets, suggesting possible cross-contamination (molecular typing confirmation pending). In outlet 1, an identical resistance pattern (pattern 2, serogroup D) was detected across chicken meat, cutting boards, and knives, and pattern 18 was found in meat and board samples. In outlet 2, shared resistance patterns (patterns 2, serogroup B) between isolates obtained from meat and knife samples. In outlet 3, a shared resistance pattern (patterns 12, serogroup E) was detected between isolates from the cutting board and knife surfaces. Similarly, in outlet 4, the same resistance pattern (pattern 8, serogroup C2) was observed among isolates from meat and board samples. In outlet 5, a closely related multidrug resistance pattern (pattern 10, serogroup B) was detected on chicken meat and cutting boards.

4. Discussion

Salmonella is a major cause of foodborne disease worldwide, particularly through the consumption of poultry and poultry products [3]. This study demonstrated a high prevalence of *Salmonella* spp. in chicken meat, cutting board, and knife samples collected from retail outlets of wet markets in Phatthalung, Thailand. This finding indicates that chicken retail outlets may serve as important reservoirs for *Salmonella*. This is consistent with several previous reports showing the widespread distribution of this bacterium in poultry retail products and production chains, especially in developing countries where sanitation and hygiene practices may be substandard [23, 24]. Serogroup B, closely followed by C, was identified as the most common group. This distribution is consistent with previous studies reporting a high proportion of these two serogroups among chicken-associated *Salmonella* isolates [24, 25]. Moreover, nontyphoidal *Salmonella*, including *S. Typhimurium* and *S. Enteritidis*, was detected in the samples. These serovars are well recognized as major zoonotic agents of human salmonellosis worldwide [26, 27]. Although most *Salmonella* infections are self-limiting, serovars such as *S. Enteritidis* and *S. Typhimurium* are more likely to cause invasive disease. Notably, these serovars were identified as MDR strains. This is similar to previous reports indicating the high proportion of MDR *S. Enteritidis* and *S. Typhimurium* detected in poultry and poultry products [24, 28-29]. Therefore, the presence of these serovars in retail chicken outlets poses a significant threat to food safety. Continuous monitoring of these high-risk serovars is essential to control the risk of severe foodborne outbreaks and invasive salmonellosis in the community.

Table 2. Non-multidrug resistance (pattern 1-6) and multidrug resistance profiles (pattern 7-21) of *Salmonella* spp. isolated from retail chicken outlets in Phatthalung, Thailand.

Pattern	Resistance profile	No. of antibiotic	No. of class	Serogroup	No. of isolates (%)
1	C	1	1	B	2 (4.65)
2	AMP	1	1	B, D, C2	6 (13.95)
3	AMP, S	2	2	D	1 (2.33)
4	AMP, W	2	2	B	2 (4.65)
5	AMP, TE	2	2	B	1 (2.33)
6	AMP, GEN, S	3	2	E	1 (2.33)
7	AMP, NA, S	3	3	C2	1 (2.33)
8	AMP, TE, DO, GEN, S	5	3	C2	2 (4.65)
9	AMP, TE, DO, S, W	5	4	C2	1 (2.33)
10	AMP, TE, DO, GEN, S, C	6	4	B, C1	3 (6.98)
11	AMP, NA, ENR, CIP, LEV, S	6	3	B, C1, D	3 (6.98)
12	AMP, TE, GEN, S, C, W	6	5	E, C1	3 (6.98)
13	AMP, NA, ENR, CIP, LEV, TE	6	3	B	1 (2.33)
14	AMP, CTX, NA, ENR, CIP, LEV, S	7	4	G	1 (2.33)
15	AMP, CTX, TE, GEN, S, C, W	7	6	C1	1 (2.33)
16	AMP, NA, ENR, CIP, LEV, TE, DO, S	8	4	B, C2, D	4 (9.30)
17	AMP, CTX, NA, ENR, CIP, LEV, TE, DO	8	4	C2	1 (2.33)
18	AMP, NA, ENR, CIP, LEV, TE, DO, S, W	9	5	B, E, C1	6 (13.95)
19	AMP, CTX, CTR, NA, TE, DO, S, C, W	9	7	C1	1 (2.33)
20	AMP, CTX, NA, ENR, CIP, LEV, TE, DO, S, C	10	6	G	1 (2.33)
21	AMP, CTX, CTR, NA, ENR, CIP, LEV, TE, DO, S, C, W	12	7	C1	1 (2.33)

The antimicrobial susceptibility results showed high resistance to ampicillin, streptomycin, and tetracycline. This finding is consistent with previous reports in Thailand. Chuanchuen and Padungtod [9] reported that these three antibiotics are among the most frequently resistant in *Salmonella* from swine and poultry. Furthermore, our results align with the core resistance patterns observed in retail local markets and poultry slaughterhouses [10-11], indicating the long-standing selective pressure exerted by extensive antibiotic use in the Thai poultry industry. Notably, a strong association between nalidixic acid resistance and fluoroquinolone resistance was observed; among the 20 nalidixic-resistant isolates, 90% (18/20) exhibited resistance to ciprofloxacin, enrofloxacin, and levofloxacin. These findings correspond to reported resistance levels in broilers, where nalidixic acid resistance reached 49.54% and ciprofloxacin resistance reached 23.85% [30]. Similar trends of high resistance to nalidixic acid (69.4%) and ciprofloxacin (16.7%) have also been reported in broilers at slaughterhouses in Thailand [31]. Our findings contrast with reports from regions with well-established antimicrobial regulations, such as European countries, where high ciprofloxacin susceptibility is still commonly observed [32]. Furthermore, our findings suggest a clear shift from previous reports in Thailand, where 100% susceptibility to ciprofloxacin in chicken meat was observed [10, 33-34]. This difference suggests that fluoroquinolone resistance has been increasing over the past decades. A key driver of this trend is likely the extensive use of enrofloxacin, a veterinary fluoroquinolone widely administered in Thai poultry farming for prophylaxis and treatment. Because enrofloxacin is partially metabolized to ciprofloxacin *in vivo*, its use selects for mutations in DNA gyrase (*gyrA/gyrB*) and topoisomerase IV (*parC/parE*) that confer cross-resistance to all fluoroquinolones used in human medicine. Ciprofloxacin is the drug of choice for the treatment of clinical salmonellosis. The high level of resistance observed in this study poses a significant challenge to effective patient management. On the other hand, resistance to third-generation cephalosporins (CTX and CTR) remained low compared to other antimicrobial classes. Similar results have been reported in poultry and retail chicken [13, 24, 35]. This finding suggests a low prevalence of ESBL-producing isolates. However, ESBL production should be further confirmed by combination disk testing or detection of ESBL genes. The most likely ESBL gene families in this context are *bla*CTX-M (particularly CTX-M-15 and CTX-M-55, which are prevalent in Southeast Asian poultry) and *bla*TEM. Future work should include phenotypic confirmation by combined disk testing (cefotaxime ± clavulanate) and molecular screening for these resistance genes.

Nearly 70% of isolates exhibited multidrug resistance (MDR). This observation is similar to several previous studies [12-13, 35]. The presence of MDR strains in retail chicken outlets is a public health concern because it can limit treatment options. Of particular concern, patterns 19 and 21 — each resistant to 9–12 antibiotics spanning seven antimicrobial classes — meet the criteria for extensively drug-resistant (XDR) strains. Against these isolates, chloramphenicol and third-generation cephalosporins (CTX, CTR) appear to retain some activity, as indicated by the resistance profiles observed in this study; however, treatment decisions should be guided by clinical susceptibility testing. The emergence of XDR strains in a retail food environment with no direct clinical link underscores the urgency of integrating food-chain surveillance into national antimicrobial resistance monitoring programs. The dissemination of such high-level resistance may be further facilitated by integron-mediated horizontal gene transfer, a mechanism well-documented in clinical and environmental bacterial pathogens [36]. Phenotypic-genotypic characterization approaches, as applied in related AMR studies [37], should be employed in future investigations to elucidate the specific resistance genes underlying the MDR and XDR profiles observed here. Furthermore, exploration of naturally derived antimicrobials, including insect-derived antimicrobial peptides [38], propolis extracts [39], and phytochemicals from plant sources [40], may offer complementary strategies to address the growing AMR challenge. The MAR index supported these observations, as most isolates (72.09%) had values greater than 0.2, and more than one-third exceeded 0.5, indicating contamination from high-risk sources with frequent antibiotic use [41]. Similar antimicrobial resistance patterns were found in isolates from chicken meat, cutting boards, and knives within the same outlets, suggesting cross-contamination during handling. However, further molecular typing would be required for confirmation. This finding is supported by previous studies

showing that chicken meat and contact surfaces can act as sources of *Salmonella* transmission in retail settings [23, 42]. Moreover, *S. Typhimurium*, *S. Enteritidis*, and other serovars originating from chicken have been associated with human gastrointestinal infections [43].

Overall, this study shows the presence of MDR. Several limitations of this study should be acknowledged. First, the sample size was relatively small (five outlets, 125 total samples), and sampling was confined to a single province over two months (June–July 2024), which may limit the generalizability of findings. Second, samples were collected on a single visit per outlet; repeated sampling across seasons would provide a more robust estimate of contamination prevalence. Third, the study was unable to trace isolates back to farms of origin, so the point at which resistance was acquired, farm, slaughterhouse, or retail, remains unclear. Fourth, cross-contamination was inferred solely from shared antibiogram patterns and serogroups; molecular typing methods such as PFGE or MLST are required for definitive confirmation. Finally, the boiling-lysis DNA extraction method used in Section 2.5, while practical, may yield a lower-quality template compared with commercial extraction kits, a factor that should be considered when interpreting PCR results. Despite these limitations, the study provides important baseline data on the AMR burden at the retail level in Southern Thailand. *Salmonella* in retail chicken outlets underscores the importance of proper antimicrobial use in poultry production and improved hygiene practices at retail outlets to mitigate the spread of resistant strains to consumers.

5. Conclusions

This study demonstrated that retail chicken outlets in Phatthalung, Thailand, serve as significant reservoirs of *Salmonella* spp., with a high prevalence of antimicrobial resistance. *Salmonella* spp. isolated from retail chicken outlets showed high levels of antimicrobial resistance, particularly to ampicillin (95.35%), streptomycin (69.77%), tetracycline (60.46%), and fluoroquinolones (41.86%), with a high proportion of multidrug-resistant isolates (69.77%). Serogroup B was the most frequently detected, followed by serogroups C2 and C1. Similar resistance patterns in meat and environmental samples suggested possible cross-contamination in retail settings, pending molecular confirmation. Notably, two resistance patterns (19 and 21) met the criteria for extensively drug-resistant (XDR) strains, representing a significant public health concern that warrants urgent attention. These findings underscore the need for: (i) stricter enforcement of hygiene practices at retail wet markets, including regular surface disinfection of cutting boards and knives; (ii) national surveillance programs that systematically monitor antimicrobial resistance in retail poultry; and (iii) antimicrobial stewardship interventions targeting veterinary use of fluoroquinolones and other critically important antibiotics in the Thai poultry industry.

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Declaration of AI-Assisted Writing

AI-assisted language tools were used during the preparation of this manuscript to support writing and editing. The authors are fully responsible for all scientific content, data interpretation, and conclusions presented herein.

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