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Research Article

Antimicrobial Resistance, Molecular Serotyping and Virulence Profiles of *Listeria Monocytogenes* Isolated From Raw Chicken Meat in Bangkok and Metropolitan Provinces

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ABSTRACT

This study investigated the prevalence, antimicrobial resistance, molecular serogroup, and virulence genes of *Listeria monocytogenes* isolated from raw chicken meat samples collected from traditional markets and hypermarkets in Bangkok and metropolitan provinces, Thailand. A total of 33 *L. monocytogenes* isolates were recovered from 220 samples (15%), with contamination detected in 13 of 150 samples (8.7%) from traditional markets and 20 of 70 samples (28.6%) from hypermarkets. Molecular serotyping classified 24 isolates (72.7%) into serogroup 1/2b, 3b (Division I) and 9 isolates (27.3%) into serogroup 1/2a, 3a (Division II). Among isolates from traditional markets, 4 belonged to serogroup 1/2b, 3b, while 9 were classified as serogroup 1/2a, 3a. In hypermarkets, serogroup 1/2b, 3b was predominant (18 isolates), whereas 2 isolate belonged to serogroup 1/2a, 3a. Antimicrobial susceptibility testing against 14 antibiotics revealed the highest resistance to clindamycin (25/33, 75.8%), followed by penicillin G (14/33, 42.4%), ampicillin (11/33, 33.3%) and tetracycline (5/33, 15.1%). Multidrug resistance (resistance to □ 3 antibiotic classes) was observed in 18.2% of isolates. Molecular characterization revealed all *L. monocytogenes* isolates (100%) harbored the virulence-associated genes (*inlA*, *inlC*, *actA*, *hly*A, and *iap*). Notably, 97% of the isolates contained the *inlJ* gene. Additionally, the widespread resistance to common therapeutic agents, along with the presence of serogroups 1/2b, 3b and 1/2a, 3a, raises public health concerns, as serotype 1/2b and 1/2a have been most frequently associated with human listeriosis.

Keywords: Antimicrobial resistance, Chicken meat, *Listeria monocytogenes*, Molecular serotyping, Virulence gene

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Introduction

The genus *Listeria* comprises Gram-positive, facultatively anaerobic bacteria belonging to the family *Listeriaceae*. Six species have been identified: *L. monocytogenes, L. ivanovii, L. innocua, L.welshimeri, L.seeligeri,* and *L.grayi* [1]. Among these, only *L.monocytogenes* and *L. ivanovii* have been reported to be pathogenic [2]. *L. monocytogenes* is a significant foodborne pathogen [3] and has been isolated from a wide range of food products, including raw meat, processed meat, dairy products, and vegetables [4]. Evidence suggests that meat products serve as major transmission vehicles for *L. monocytogenes*, posing a direct risk to consumers.

L. monocytogenes is classified into four phylogenetic evolutionary lineages (I, II, III, and IV). Serotyping is widely used to characterize *L. monocytogenes* based on cell wall and flagellar antigens [5, 6]. Lineage I of *Listeria monocytogenes* comprises serotypes 1/2b, 3b, 3c, and 4b, while lineage II includes serotypes 1/2a, 1/2c, and 3a. Lineages III and IV consist of serotypes 4a and 4c [7]. Although 13 serotypes have been identified, serotypes 1/2a, 1/2b, and 4b are the most frequently isolated in human listeriosis cases, with serotype 4b being the primary cause of epidemic outbreaks [6, 8, 9]. Notably, serotype 1/2a is highly prevalent in raw food products and ready-to-eat (RTE) foods, highlighting the potential utility of serotyping as a virulence screening tool.

The pathogenicity of *L. monocytogenes* is associated with several virulence factors, including actin assembly-inducing protein (*actA*), internalins (*inlA, inlB, inlC, and inlJ*), invasion-associated protein (*iap*), listeriolysin O (*hlyA*), and phosphatidylinositol phospholipase C (*plcA*) [7, 10, 11]. The virulence genes *actA*, *iap*, *hlyA*, and *inlA* play critical roles in *L. monocytogenes* pathogenicity. *ActA* gene enables bacterial motility within host cells by inducing actin polymerization. *Iap* gene aids in bacterial cell division and promotes host cell invasion. *HlyA* gene encodes listeriolysin O, which allows the bacterium to escape from the phagosome into the cytosol. *Inl* genes facilitate bacterial entry into host cells by binding to E-cadherin on epithelial cells. These virulence factors are crucial to the bacterium's pathogenicity and contribute to severe human infections.

Antibiotic resistance in *L. monocytogenes* has been widely reported with resistant isolates found in ready-to-eat foods, raw foods, environmental sources and sporadic cases of human listeriosis [12]. A previous survey of pathogens in food samples from supermarkets and open markets in Bangkok and its surrounding provinces reported the presence of *L. monocytogenes* contamination [13]. Among these food products, raw meat collected from markets was found to harbor *L. monocytogenes*, with an incidence rate ranging from 3% in open markets to 10% in supermarkets [13]. Given the existing data on antimicrobial resistance in Thailand, the emergence of multidrug-resistant *L. monocytogenes* could pose a significant public health concern. Despite this, few studies in Thailand have reported on the antimicrobial resistance of *L. monocytogenes* isolated from raw meats in Bangkok markets [14, 15]. The present study aimed to characterize *L. monocytogenes* strains isolated from chicken meats in markets in Bangkok and the metropolitan provinces through molecular subtyping, assess their antimicrobial susceptibilities, and examine their virulence gene profiles. Regular monitoring of its prevalence in food materials, particularly meats sold

in markets, along with characterization of its drug susceptibility and genotypes, is essential for disease surveillance.

Materials and methods

Sample collection, isolation and identification of L. monocytogenes

A total of 220 raw chicken carcasses were randomly collected from traditional markets and hypermarkets in Bangkok and the surrounding metropolitan provinces between January 2018 and December 2022. The surrounding metropolitan area comprised four provinces: Nonthaburi, Pathum Thani, Samut Prakan, and Samut Sakhon. Of these, 150 fresh chicken meat samples were obtained from traditional markets, while 70 were sourced from hypermarkets. All samples were transported on ice to the laboratory within 24 hours and stored at 4°C until analysis.

L. monocytogenes detection followed the ISO 11290-1:2014 protocol [16]. To begin, 25 g of each sample was pre-enriched in 225 milliliters of Half Fraser broths at 30°C for 24 hours, followed by secondary selective enrichment in Half Fraser broth supplemented with Half Fraser supplement (Oxoid, UK) at 37°C for 24 hours. A 10 µL of the enriched culture was then streaked onto Chromogenic Listeria Agar (ISO) (CM1084; Oxoid). After incubation incubated at 37°C for 24 hours, the plates are inspected for colonies. *L. monocytogenes* colonies typically appear as turquoise blue with opaque white halos surrounding them. Presumptive *L. monocytogenes* colonies were re-streaked on tryptone soya yeast glucose agar. Identification was confirmed through Gram staining, haemolytic activity on sheep blood agar, carbohydrate utilization patterns (mannitol, rhamnose and xylose), tumbling motility, oxidase and catalase reaction.

***L. monocytogenes* confirmation and serogroup identification by PCR**

A 10-mL aliquot of bacterial culture was used for DNA purification with the Wizard Genomic DNA Purification Kit (Promega, USA). The L1/U1 primers were employed to target the *16S rRNA* gene (938 bp) for the identification of the *Listeria* genus, following the PCR conditions outlined by Border et al. [17]. To confirm *L. monocytogenes* isolates, the LMplcF/LMplcR primers were used to amplify the *plc* gene (231 bp), a species-specific marker, according to the PCR conditions described by Wachiralurpan et al. [18].

The identification of *L. monocytogenes* serogroups involved the parallel analysis of somatic antigens, flagellar antigens, and virulence genes, which classify *L. monocytogenes* strains into distinct serogroups, as described by Borucki & Call [19], Jinneman & Hill [20] and Zhang et al. [21]. The primer sets for molecular subtyping and annealing temperature for PCR are described in Table 1. All PCR reactions were performed in a 25-µL final volume, containing 1X GoTaq® Colorless Master Mix (Promega, USA), 1 µM of each forward and reverse primer, and 50 ng of DNA template. The PCR mixture was processed using the T100 Thermal Cycler (BioRad, USA) under the conditions recommended in the GoTaq® Colorless Master Mix (Promega, USA) protocol. The amplified products

were analyzed by 1% agarose gel electrophoresis and SYBR safe DNA gel stain. The DNA bands were observed under an ultraviolet (UV) transilluminator.

D1 and D2 primers distinguished *L. monocytogenes* serogroups into two divisions. Division I includes serotypes 1/2b, 3b, and all serotype 4 strains, while division II consists of serotypes 1/2a, 1/2c, 3a, and 3c. To further classify serotypes 1/2b, 3b, and all serotype 4 strains, GLT primers were used. FlaA primers were used to subtypes serotypes 1/2a, 3a, 1/2c, and 3c by differentiating serotypes 1/2a and 3a from serotypes 1/2c and 3c. Additionally, LM4B primers identified serotypes 4a and 4c. This molecular subtyping assay classifies isolates into five major serogroups, with each serogroup encompassing multiple serotypes. These include serogroups 1/2a and 3a, serogroups 1/2c and 3c, serogroups 4b, 4d, and 4e, serogroups 1/2b and 3b, and serogroups 4a and 4c.

Phenotypic detection of antimicrobial resistance in L. monocytogenes isolates

Antibiotic susceptibility testing was performed using the disk diffusion method, following CLSI guidelines [22]. The tests were conducted on Mueller Hinton agar (Oxoid, UK) supplemented with 5% defibrinated sheep blood. The interpretation of results was based on the resistance breakpoints for *Staphylococcus* and *Enterococcus* species, as no specific resistance criteria for *Listeria* susceptibility testing are provided in the CLSI guidelines [23]. The following antibiotic agents were tested: ampicillin (30 μg), amoxicillin/clavulanic acid (20/10 μg), chloramphenicol (30 μg), clindamycin (2 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), erythromycin (15 μg), gentamycin (10 μg), penicillin G (10 μg), rifampicin (5 μg), tetracycline (30 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), vancomycin (30 μg), and meropenem (10 μg). *E. coli* ATCC25922 was used as a control strain.

Virulent genes of L. monocytogenes isolate identification

After confirming the *L. monocytogenes* isolates through molecular serogroup identification, the virulence gene markers (*actA, iap, hlyA, inlA, inlC*, and *inlJ*) were determined using PCR, following the protocols described by Liu et al. [24], Paziak-Domanèska et al. [25] and Kalorey et al. [26] (Table 1).

Results

Samples were collected from traditional markets in Bangkok and nearby provinces. A total of 150 raw chicken meat samples were obtained from traditional markets, while 70 samples were purchased from hypermarkets, resulting in 220 samples analyzed for *L. monocytogenes* contamination. Approximately five suspected colonies were isolated from each selective differential plate and confirmed by PCR. Among the 220 raw chicken meat samples, 33 (15.0%) tested positive for *L. monocytogenes*, as confirmed by biochemical tests and PCR. The pathogen was detected in 13 of the 150 samples (8.7%) from traditional markets and in 20 of the 70 samples (28.6%) from modern trade sources.

Molecular serotyping identified two serogroups among the 33 *L. monocytogenes* isolates: division I (1/2b, 3b) and division II (1/2a, 3a) (Table 2). Serogroup 1/2b, 3b was the most prevalent, detected in 24 of the 33 isolates (72.7%), followed by serogroup 1/2a, 3a, found in 9 isolates (27.3%).

Notably, serogroup 1/2b, 3b was the dominant serogroup in hypermarkets samples, with 18 of the 20 isolates belonging to this group.

Table 1 Primer sets for the molecular serotype detection and virulent genes characterization of *L. monocytogenes* isolates.

Primer set	Primer sequences (5□–3□)	Product size (bp)	Annealing Tm (°C)	Target specificity	Ref.
D1	F:CGATATTTTATCTACTTTGTCA R:TTGCTCCAAAGCAGGGCAT	214	59	Division I (serotype 1/2b, 3b, 4b, 4d, 4e, 4a, 4c)	[19]
D2	F:GCGGAGAAAGCTATCGCA R:TTGTTCAAACATAGGGCTA	140	59	Division II (serotype 1/2a, 1/2c, 3a, 3c)	
FlaA	F:TTACTAGATCAAACTGCTCC R:AAGAAAAGCCCCTCGTCC	538	54	Serotype 1/2a, 3a	
GLT	F:AAAGTGAGTTCTTACGAGATTT R:AATTAGGAAATCGACCTTCT	483	45	Serotype 1/2b, 3b	
LM4B	F:CAGTTGCAAGCGCTTGGAGT R:GTAAGTCTCCGAGGTTGCAA	268	55	Serotype 4a, 4c	[20]
inlA	F:ACGAGTAACGGGACAAATGC R:CCCGACAGTGGTGCTAGATT	800	55	Internalin	[10]
inlB	F:TGGGAGAGTAACCCAACCAC R:GTTGACCTTCGATGGTTGCT	884	55		
inlC	F:AATTCCCACAGGACACAACC R:CGGGAATGCAATTTTTCACTA	517	55		
inlJ	F: TGTAACCCCGCTTACACAGTT R: AGCGGCTTGGCAGTCTAATA	238	55		
hlyA	F:GCAGTTGCAAGCGCTTGGAGTGAA R:GCAACGTATCCTCCAGAGTGATCG	456	60	Listeriolysin	[24]
iap	F:ACAAGCTGCACCTGTTGCAG R:TGACAGCGTGTGTAGTAGCA	131	60	Invasion-associated protein	[26]
actA	F:CGCCGCGGAAATTAAAAAAAGA R:ACGAAGGAACCGGGCTGCTAG	839	60	Assembly-inducing protein	[26]

Table 2 The results of amplified genes for *L. monocytogenes* confirmation and identification of molecular serogroup by PCR.

Source	Isolate code	L1/U1 and LMplcF/LMplcR primer	Positive of D1 primer		Positive of D2 primer	Molecular serogroups
			GLT primer	*LM4B* primer	*FlaA* primer	
Traditional market	BK/14997-014	+	-	-	+	1/2a, 3a
	BK/18317-015	+	+	-	-	1/2b, 3b
	BK/14997-012	+	+	-	-	1/2b, 3b
	BK/16199-012	+	+	-	-	1/2b, 3b
	BK/14997-005	+	-	-	+	1/2a, 3a
	BK/18317-011	+	+	-	-	1/2b, 3b
	BK/14997-013	+	-	-	+	1/2a, 3a
	BK/18317-010	+	-	-	+	1/2a, 3a
	BK/13773-005	+	-	-	+	1/2a, 3a
	BK/16199-002	+	-	-	+	1/2a, 3a
	BK/02107-009	+	-	-	+	1/2a, 3a
	BK/02107-013	+	+	-	-	1/2b, 3b
	BK/02107-019	+	+	-	-	1/2b, 3b
Hyper market	BK/26190-002	+	+	-	-	1/2b, 3b
	BK/26190-003	+	+	-	-	1/2b, 3b
	BK/26190-008	+	+	-	-	1/2b, 3b
	BK/26190-009	+	+	-	-	1/2b, 3b
	BK/26190-010	+	+	-	-	1/2b, 3b
	BK/26190-011	+	+	-	-	1/2b, 3b
	BK/27219-020	+	+	-	-	1/2b, 3b
	BK/27219-015	+	+	-	-	1/2b, 3b
	BK/27219-016	+	+	-	-	1/2b, 3b
	BK/00808-001	+	+	-	-	1/2b, 3b
	BK/00808-011	+	+	-	-	1/2b, 3b
	BK/00808-009	+	-	-	+	1/2a, 3a
	BK/00808-003	+	-	-	+	1/2a, 3a
	BK/00808-010	+	+	-	-	1/2b, 3b
	BK/03311-010	+	+	-	-	1/2b, 3b
	BK/03311-009	+	+	-	-	1/2b, 3b
	BK/03311-013	+	+	-	-	1/2b, 3b
	BK/03311-014	+	+	-	-	1/2b, 3b

Table 2 The results of amplified genes for *L. monocytogenes* confirmation and identification of molecular serogroup by PCR. *(cont.)*

Source	Isolate code	L1/U1 and LMplcF/LMplcR primer	Positive of D1 primer		Positive of D2 primer	Molecular serogroups
			GLT primer	*LM4B* primer	*FlaA* primer	
	BK/02107-001	+	+	-	-	1/2b, 3b
	BK/02107-004	+	+	-	-	1/2b, 3b

+, PCR positive result -, PCR negative result

The most frequently observed antibiotic resistance was to clindamycin (25 isolates, 75.8%), followed by penicillin G (14 isolates, 42.4%), ampicillin (11 isolates, 33.3%), tetracycline (5 isolates, 15.1%), trimethoprim/sulfamethoxazole (2 isolates, 6.1%), gentamicin (1 isolate, 3.0%), and rifampin (1 isolate, 3.0%) (Table 3).

Table 3 Antimicrobial susceptibility testing of 33 *L. monocytogenes* isolated from raw chicken meat.

Class	Antibiotic (μg)	Breakpoints (mm)			No. of isolates (%)		
		R	I	S	R	I	S
Penicillin	AMP (30 μg)	□ 28	-	□ 29	11 (33.3)	0 (0)	22 (66.7)
	PEN (10 μg)	□ 28	-	□ 29	14 (42.4)	0 (0)	19 (57.6)
β-lactam/ β-lactamase inhibitor combination	AMC (20/10 μg)	□ 13	14-17	□ 18	0 (0)	0 (0)	33 (100.0)
Fluoroquinolone	LEV (5 μg)	□ 15	16-18	□ 19	0 (0)	0 (0)	33 (100.0)
	CIP (5 μg)	□ 15	16-20	□ 21	0 (0)	0 (0)	33 (100.0)
Tetracycline	TET (30 μg)	□ 14	15-18	□ 19	5 (15.1)	2 (6.1)	26 (78.8)
Glycopeptide	VAN[a] (30 μg)	□ 14	15-16	□ 17	0 (0)	0 (0)	33 (100.0)
Aminoglycoside	GEN (10 μg)	□ 12	13-14	□ 15	1 (3.0)	0 (0)	32 (97.0)
Phenicol	CHL (30 μg)	□ 12	13-17	□ 18	0 (0)	0 (0)	33 (100.0)
Folate pathway inhibitor	SXT (1.25/23.75 μg)	□ 10	11-15	□ 16	2 (6.1)	0 (0)	31 (93.9)
Carbapenem	MEM (10 μg)	□ 19	20-22	□ 23	0 (0)	0 (0)	33 (100.0)
Rifamycin	RIF (5 μg)	□ 16	17-19	□ 20	1 (3.0)	0 (0)	32 (97.0)
Lincosamide	CLI (2 μg)	□ 14	15-20	□ 21	25 (75.8)	8 (24.2)	0 (0)
Macrolide	ERY (15 μg)	□ 13	14-22	□ 23	0 (0)	0 (0)	33 (100.0)

S, Susceptible; I, intermediate; R, resistant.

[a] Breakpoint for *Entercococcus* spp.

PEN, Penicillin G; AMP, Ampicillin; AMC, Amoxicillin-calvulanate; LEV, Levofloxacin; CIP, Ciprofloxacin; TET, Tetracycline; VAN, Vancomycin; GEN, Gentamycin; CHL, Chloramphenicol; SXT, Trimethoprim/sulfamethoxazole; MEM, Meropenem, RIF, Rifampin; CLI, Clindamycin; ERY, Erythromycin.

Multidrug resistance (resistance to □ 3 antibiotic classes) was detected in six isolates (18.2%), with the most common multidrug resistance phenotype including resistance to ampicillin, penicillin G, clindamycin, and tetracycline (Table 4). Among these multidrug-resistant isolates, four isolates belonging to serogroup 1/ 2a, 3a were recovered from both traditional and hypermarkets, while two isolates from serogroup 1/2b, 3b were obtained from hypermarkets. The highest level of antimicrobial resistance was observed in a single serogroup 1/ 2a, 3a isolate from a traditional market, which was resistant to seven antibiotics (AMP-CLI-GEN-PEN-RIF-SXT-TET). Additionally, one isolate from serogroups 1/2a and 3a, obtained from both traditional and hypermarkets, exhibited resistance to four antibiotics.

Table 4 Molecular subtype and antimicrobial resistance patterns of 33 *Listeria monocytogenes* isolated from raw chicken meat in traditional markets and hypermarkets.

Source	Serogroup	No. of isolates (%)	Antimicrobial resistance pattern (No. of isolate)
Traditional market	1/2b, 3b	6 (18.2)	CLI (3)
			CLI-PEN (3)
	1/2a, 3a	7 (21.2)	Susceptible (2)
			AMP-CLI (1)
			AMP-PEN (1)
			CLI-PEN (1)
			AMP-CLI-PEN-SXT (1)
			AMP-CLI-GEN-PEN-RIF-SXT-TET (1)
Hypermarket	1/2b, 3b	18 (54.5)	Susceptible (3)
			AMP (1)
			CLI (6)
			CLI-PEN (2)
			AMP-CLI (1)
			AMP-CLI-PEN (3)
			CLI-PEN-TET (1)
			AMP-CLI-PEN-TET (1)
	1/2a, 3a	2 (6.1)	CLI-TET-VAN (1)
			CLI-PEN-TET-VAN (1)

PEN, Penicillin G; AMP, Ampicillin; TET, Tetracycline; VAN, Vancomycin; GEN, Gentamycin; SXT, Trimethoprim/sulfamethoxazole; RIF, Rifampin; CLI, Clindamycin; ERY, Erythromycin.

The 33 *L. monocytogenes* isolates were examined for the presence of virulence-associated genes (Table 5). The *inlA*, *inlC*, *actA*, *hlyA* and *iap* genes were detected in 9 isolates of serogroup 1/2a, 3a, and 24 isolates of serogroup 1/2b, 3b. However, the *inlJ* gene was absent in one isolate from serogroup 1/2b, 3b.

Table 5 Virulence gene detection in *L. monocytogenes* isolates from chicken meat.

Serogroup	Virulence gene	Number of positive isolates (%)
1/2a, 3a	*Iap+actA+hlyA-inlA+inlC+inlJ*	9/9 (100.0)
1/2b, 3b	*Iap+actA+hlyA+inlA+inlC+inlJ*	23/24 (95.8)
	Iap+actA+hlyA+inlA+inlC	1/24 (4.2)

Discussion

This study highlights the significant prevalence of *L. monocytogenes* contamination in raw chicken meat sold in Thai markets. Detection rates were 8.7% in traditional markets and 28.6% in hypermarkets, both exceeding those reported in previous studies conducted in Thailand [13]. The higher prevalence in hypermarkets aligns with earlier findings [14] and is consistent with Goh et al. (2012) [27]. In contrast, a lower contamination rate (2.5%) has been reported for frozen chicken meat in Thailand [28], reinforcing the observation that frozen chicken meat generally harbors lower contamination levels than fresh or ready-to-eat (RTE) chicken products.

The higher incidence of *L. monocytogenes* in hypermarkets may be attributed to prolonged refrigeration, which supports bacterial survival and proliferation, as *L. monocytogenes* thrives at low temperatures [29]. Meanwhile, fresh chicken meat in traditional markets is typically exposed to ambient temperatures (30–37°C), an optimal range for bacterial growth. Nonetheless, the lower prevalence observed in traditional markets compared to hypermarkets may be influenced by factors such as higher turnover rates, shorter storage durations, and different handling practices. These findings emphasize the need for proper food handling and cooking practices to reduce the risk of foodborne infections.

Studies from other regions have reported even higher levels of *L. monocytogenes* contamination in raw poultry products. For instance, *L. monocytogenes* was detected in 29.6% of samples in Korea [30], 25.71% in Malaysia [27], 38.2% in northern Greece [31], 24.5% in Italy [32], and 22% in the Nordic countries [33]. Thus, the incidence of *L. monocytogenes* in raw chicken meat in Thailand is comparable to or lower than that reported in other Asian and European countries. Variations in prevalence may be attributed to differences in contamination levels during slaughter, processing, and carcass handling.

Molecular serotyping of raw chicken meat identified two distinct serotype patterns: 1/2b and 3b, as well as 1/2a and 3a. The prevalence of isolates belonging to serogroups 1/2b and 3b was higher than that of serogroups 1/2a and 3a, consistent with a previous review study [34]. However, conventional serotyping procedures are necessary to accurately confirm serotype identity and determine the true prevalence. Although all strains of *L. monocytogenes* are considered potential pathogens, the serotypes most commonly associated with foodborne listeriosis are 1/2a, 1/2b, and 4b, with serotype 4b notably implicated in listeriosis outbreaks [35, 36]. These findings suggest that contaminated meat may act as a vehicle for transmitting virulent *L. monocytogenes* to humans.

The results of this study indicate a high incidence of antimicrobial resistance in *L. monocytogenes* strains isolated from raw meat products in Thailand. Among the isolates, 20 (75.8%) were resistant to clindamycin, followed by 13 (42.4%) resistant to penicillin. The high prevalence of clindamycin resistance observed in this study aligns with previous reports [37]. However, this resistance level remains lower than that reported in studies where penicillin resistance approaches 90% [38]. Six *L. monocytogenes* strains (18.2%) exhibited multidrug resistance, all resistant to ampicillin, penicillin, tetracycline, and clindamycin. Notably, serogroups 1/2a and 3a displayed the highest resistance, with isolates resistant to seven antibiotics. The emergence of antimicrobial resistance in these strains may be associated with the overuse of antibiotics in animal feed and their use as secondary treatments for human infections [38]. *L. monocytogenes* acquires antimicrobial resistance through mechanisms such as conjugation, self-transferable plasmids, and both vertical and horizontal gene transfer [39, 40].

This study examined *L. monocytogenes* isolates from raw chicken meat for the presence of major internalin genes, as surface-associated internalin is recognized as playing a crucial role in the pathogenesis of listeriosis [41]. The results align with those of previous studies, where these internalin genes were found in nearly all *L. monocytogenes* isolates human listeriosis cases [42], various foods [43], and environmental samples [44]. In our study, the *inlA* and *inlC* genes were detected in all *L. monocytogenes* isolates, while the *inlJ* gene was observed in 32 (97%) of the isolates. Additionally, the assembly-inducing protein (*actA* gene), invasion-associated protein (*iap* gene), and listeriolysin O (*hlyA* gene) were detected in almost all isolates recovered from meat samples. The presence of these virulence-associated genes is consistent with previous findings [45, 46]. The detection of these six virulence-associated genes in nearly all isolates suggests that these *L. monocytogenes* strains could potentially be virulent.

Conclusions

This study highlights the significant prevalence of *L. monocytogenes* contamination in raw chicken meat from Bangkok and surrounding provinces, with a higher detection rate in hypermarkets (28.6%) compared to traditional markets (8.7%). Molecular serotyping revealed two dominant serogroups, with serogroup 1/2b, 3b being the most prevalent, particularly in hypermarkets. High levels of antimicrobial resistance were observed in *L. monocytogenes* isolates of serogroup 1/2a, 3a. The presence of key virulence genes (*inlA, inlC, inlJ, actA, iap*, and *hlyA*) in nearly all isolates indicates their pathogenic potential. Future studies should focus on whole-genome sequencing to better understand the genetic diversity and transmission dynamics of *L. monocytogenes* in the poultry supply chain.

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