RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2025.089

ScienceAsia 51 (5): 2025: ID 2025089: **1–8**

Molecular detection of virulence genes and antibiotic resistance profiles in *Salmonella* spp. contaminated on food-contact surfaces in restaurant settings

Jintana Wongwigkarn[a], Siriwat Kucharoenphaibul[a], Sutthirat Sitthisak[a], Rapee Thummeepak[a], Rattiya Cheewapat[a], Kunsuda Nimanussornkul[b], Supat Khongfak[c], Kannipa Tasanapak[a,*]

[a] Department of Microbiology and Parasitology, Faculty of Medical Sciences, Naresuan University, Phitsanulok 65000 Thailand

[b] Community Economics Development and Research Centre, Faculty of Economics, Chiang Mai University, Chiang Mai 50200 Thailand

[c] Office of Disease Prevention and Control Region 3 Nakhon Sawan, Department of Disease Control, Ministry of Public Health, Nakhon Sawan 60000 Thailand

[*]Corresponding author, e-mail: kannipal@nu.ac.th

Received 20 Oct 2024, Accepted 18 Oct 2025
Available online 31 Oct 2025

ABSTRACT: This study investigates the prevalence, virulence genes, and antimicrobial resistance profiles of *Salmonella* spp. isolated from food contact surfaces and food handlers in local restaurants in northern Thailand. *Salmonella*, a prominent foodborne pathogen, can thrive under diverse conditions, leading to the contamination of various food products and causing illnesses. It is acknowledged as a prominent foodborne pathogen. A total of 650 samples were collected, resulting in the identification of 21 *Salmonella* belonging to serogroups B (52.38%), D (14.30%), A, C1, E1 (each 9.52%), and C2 (4.76%). Virulence gene analysis revealed that *Salmonella* group B isolates possessed the highest number of virulence genes, with up to 12 per isolate, including commonly detected genes such as *msg*A, *sop*B, and *sif*A. A significant correlation between specific virulence genes and antibiotic resistance was observed. Of the *Salmonella* isolates recovered in this study, 43% were identified as multidrug-resistant (MDR). Among these MDR strains, 78% were isolated from the hands of food handlers, while the remaining originated from kitchen surfaces. The combined threat posed by these *Salmonella* strains is underscored by the presence of multiple virulence genes and elevated levels of antibiotic resistance. Effective control measures, including stringent hygiene practices, regular cleaning and sanitization of kitchen equipment, and the implementation of Hazard Analysis and Critical Control Points (HACCP) systems, are essential to minimize contamination risk and prevent the spread of harmful pathogens. Ongoing surveillance and antimicrobial resistance monitoring programs are crucial for managing the threat of MDR *Salmonella* in restaurant environments.

KEYWORDS: *Salmonella* spp., antibiotic resistance, virulence gene, food safety, foodborne pathogens

INTRODUCTION

Salmonella spp. are significant foodborne pathogens that cause gastrointestinal infections, leading to symptoms such as diarrhea, vomiting, abdominal pain, and, in severe cases, systemic infections, particularly among vulnerable populations such as children, the elderly, and immunocompromised individuals [1, 2]. There have been reports of *Salmonella* spp. found in Thailand in various foods, including meat, seafood, and fermented pork [3–5], causing food poisoning outbreaks [6]. Contamination can occur at various stages of the food handling chain, from preparation to serving. In pork products, pathogenic microorganisms may lead to the accumulation of biogenic amines, which compromise both food safety and product quality, although appropriate packaging has been shown to reduce spoilage and extend shelf life [7]. Unsanitary food contact surfaces such as cutting boards, knives, and dishes have been recognized as potential sources of cross-contamination [8, 9]. However, one area that remains under-investigated is the role of food handlers' hands in transmitting *Salmonella* within restaurant environments, despite being a critical control point in food safety protocols. The detection of *Salmonella* from bare hands is particularly concerning, as this reflects poor hygiene and represents a direct route of transmission to ready-to-eat food. Equally concerning is the increasing detection of *Salmonella* strains exhibiting antimicrobial resistance. Multidrug-resistant (MDR) *Salmonella* complicates treatment and increases the risk of severe outcomes, posing a major public health challenge [10–13]. Surveillance data from northern Thailand, where food hygiene enforcement in small restaurants may be limited, remain sparse. This lack of localized data hinders effective interventions. Moreover, the detection of virulence genes in *Salmonella* spp. isolated from food contact surfaces adds another layer of importance. The virulence genes of *Salmonella* play a crucial role in its pathogenicity, as they are located on *Salmonella* pathogenicity islands (SPI), plasmids, or the genome. These genes are involved in cell invasion, intracellular survival, and colonization, ultimately increasing the bacterium's

capacity to cause severe disease [14, 15]. A PCR-based method is employed to develop a technique for identifying *Salmonella* spp. by targeting the highly conserved *inv*A gene, which is universally recognized as the standard for *Salmonella* genus identification [16, 17]. Researchers subsequently distinguished the serogroups using additional serogroup-specific primers [18, 19]. Surveillance of antimicrobial resistance patterns in *Salmonella* isolates from restaurant environments is essential for understanding the spread of resistant strains and informing effective treatment strategies. Identifying these genes helps in assessing the potential risk posed by specific *Salmonella* strains and in understanding the mechanisms of their pathogenicity.

This study addresses a key gap by investigating *Salmonella* contamination on food contact surfaces in restaurants in northern Thailand, with particular emphasis on contamination from food handlers' hands. In addition, it examines the presence of virulence genes and antimicrobial resistance profiles in the isolates. These findings are essential for improving food safety practices and guiding public health interventions in the region.

MATERIALS AND METHODS

Sample collection and identification of *Salmonella* spp.

Three sets of samples were collected from various sources and categorized into three distinct groups: the first group (G1) were from the kitchen areas, including the surface of 100 chopping boards and 100 knives; the second group (G2) were from individuals, specifically, the hands of 75 food handlers and the hands of 75 butchers in restaurant settings; and the third group (G3) from the dining areas, encompassing the surface of 100 dishes, 100 spoons, and 100 glasses within the restaurants. Swab samples were transferred into 0.1% peptone water (Oxoid, UK). Each sample was incubated at 37 °C for 18 ± 2 h. This enrichment culture was inoculated into a selective enrichment medium for *Salmonella* species; 1 ml was inoculated in 10 ml of Rappaport-Vassiliadis Soya broth (RVS, Oxoid) and incubated at 41.5 °C for 24 h. The positive results from RVS were inoculated on Xylose Lysine Deoxycholate Agar (XLD agar, Oxoid), observed for typical *Salmonella* spp. colonies. Selected colonies were further tested using standard biochemical assays [20], including glucose fermentation, negative urease activity, lysine decarboxylation, negative indole reaction, and hydrogen sulfide (H_2S) production. *Salmonella* Typhimurium DMST 423 was used as the positive control strain and exhibited the expected biochemical profile: negative for indole and urease, but positive for glucose fermentation, lysine decarboxylation, and H_2S production. Final confirmation of *Salmonella* isolates was performed using PCR.

Molecular confirmation of *Salmonella* spp.

PCR confirmation of a putative *Salmonella* isolate via detected *inv*A is a common molecular target for *Salmonella*. *S.* Typhimurium DMST 423 was used as the positive control strain for PCR test. *E. coli* ATCC25922 was used as negative control strain for PCR specificity tested. The sequences of forward and reverse primers are 5′-GTGAAATTATCGCCACGT TCGGGCAA-3′ and 5′-TCATCGCACCGTCAAAGGAAC C-3′, respectively [16]. PCR amplifications were performed in a final volume of 25 µl containing 2 µl of DNA template, 20 pmol of each primer, 12.5 µl of OnePCR™ mixture (GeneDireX, Inc., Taoyuan, Taiwan), and sterile distilled water. The amplification started with a pre-denaturation step at 94 °C for 10 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 1 min, and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min. The PCR products were analyzed by 1% agarose gel electrophoresis.

PCR amplification for *Salmonella* serotyping

Serotyping of the *Salmonella* spp. was performed by PCR amplification. The sets of primers used in this study are listed in Table S1, which includes primers for O-serogrouping, H-typing, and dT-fermentation. O-grouping in this study identified *Salmonella* serogroups A to E based on primers targeting the genes *prt*, *rfb*J, *wzx*C1, *wzx*C2, *tyv*, and *wzx*E. H-typing identified phase I H types a, b, and d. The third set of primers was used to determine d-tartrate (dT) fermentation for identifying serovar Paratyphi B biovar Java. A primer targeting the *ori*C region was included in each PCR reaction to ensure that a negative result was not due to PCR failure. The PCR products were subsequently analyzed using agarose gel electrophoresis.

Detection of virulence genes

To predict the virulence potential of *Salmonella* spp., sixteen virulence genes were tested in all 21 isolates by PCR detection following Skyberg et al [22] with some modifications. The list of these primer sets is shown in Table S2. PCR reactions were performed in a total volume of 25 µl containing 2 µl of DNA template, 20 pmol of each forward and reverse primer, 12.5 µl of OnePCR™ mixture, and sterile distilled water to a final volume of 25 µl. The PCR cycle started with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s for all primer sets (except *pef*A at 60 °C), extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. PCR products were analyzed by 1% agarose gel electrophoresis.

Antimicrobial susceptibility test

All *Salmonella* spp. isolates were assessed for their antibiotic resistance pattern using the Kirby-Bauer agar

disc diffusion method on Mueller Hinton agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India) to determine the susceptibility of *Salmonella* spp. as guided by the CLSI guidelines (2025) [23]. Fourteen antibiotics (Oxoid) used in this study were categorized into eight classes: class 1, penicillins: piperacillin/-tazobactam (PTZ; 100/10 µg) and ampicillin (AMP; 10 µg); class 2, cephems: third-generation cefotaxime (CTX; 30 µg), ceftazidime (CAZ; 30 µg), and ceftriaxone (CRO; 30 µg), and fourth-generation cefepime (FEP; 30 µg); class 3, carbapenems: imipenem (IPM; 10 µg) and meropenem (MEM; 10 µg); class 4, fluoroquinolones/quinolones: ciprofloxacin (CIP; 5 µg) and nalidixic acid (NA; 30 µg); class 5, macrolides: azithromycin (AZM; 15 µg); class 6, phenicols: chloramphenicol (C; 30 µg); class 7, folate pathway antagonists: trimethoprim/sulfamethoxazole (SXT; 1.25/23.75 µg); and class 8, tetracyclines: tetracycline (TE; 30 µg). An organism was defined as MDR if it was non-susceptible to at least one agent in three or more antimicrobial categories.

Statistical analysis

The association between virulence genes and the antimicrobial resistance profile of *Salmonella* spp. was investigated using Pearson's correlation analysis. The sample size was 21, and the degree of freedom was 1. The correlation was considered statistically significant when the p-value was less than 0.05 (p-value < 0.05).

RESULTS

Salmonella spp. detection and serotyping

Out of 650 swab samples collected from various restaurants, 28 presumptive *Salmonella* isolates were identified, of which 21 were confirmed positive for *Salmonella* through *inv*A gene detection by PCR. The highest contamination was observed on the hands of restaurant workers, accounting for 66.7% (14 out of 150 hand samples). This was followed by the kitchen environment, where five isolates were detected on chopping boards and knives, representing 23.8% of the total contamination. Additionally, two isolates were found on spoons in the dining area (9.5%).

Serotyping results revealed the presence of multiple *Salmonella* serogroups. Specifically, three *Salmonella* serovar Typhi strains were detected using *prt*, *tyv*, and *vi* primers (D group), with positive identification for flagella antigen-d. Two isolates were identified as *Salmonella* serovar Paratyphi A using *prt* and flagella antigen-a primers, and eleven isolates were confirmed as *Salmonella* serovar Paratyphi B strains, producing PCR products with the *rfb*J primer (B group), flagella antigen-b positive, and dT-fermentation positive. Additionally, serogroups C1, C2, and E1 were detected in two, one, and two isolates, respectively. The distribution of *Salmonella* serogroups is summarized in Table 1.

Distribution of virulence genes

A total of 21 *Salmonella* isolates obtained from the kitchen areas (G1), individuals (G2), and dining areas (G3) in restaurants were tested for virulence genes. Sixteen virulence genes were assessed, including genes located on *Salmonella* pathogenicity islands (SPI), virulence plasmids (pSLT), and other genomic regions. Twelve virulence genes (*pag*C, *sip*B, *spi*A, *msg*A, *prg*H, *spa*N, *org*A, *sit*C, *lpf*C, *sif*A, *sop*B, and *inv*A) are located on SPI; *spv*B and *pef*A are carried by plasmid; *tol*C, *iro*N, and *cdt*B are in the bacterial genome. Notably, *msg*A, *sop*B, and *sif*A were among the most frequently detected genes, with *msg*A present in 57.1% of all isolates. Isolates from the kitchen environment (G1), particularly from chopping boards, exhibited the highest number of virulence genes, with up to 12 genes detected per isolate. Fig. 1 illustrates the distribution of these virulence genes across different sample sources.

Antimicrobial susceptibility test

The antibiotic resistance profiles of the 21 *Salmonella* isolates revealed that all were resistant to at least one antibiotic (Table 2). Remarkably, none of the isolates exhibited resistance to cefepime, ceftazidime, ceftriaxone, imipenem, or meropenem. However, 43% (9 isolates) were classified as MDR, with resistance to three or more antimicrobial categories. The majority of these MDR isolates (78%) were obtained from hand samples, underscoring the critical role of human vectors in the dissemination of resistant strains.

Correlation between virulence genes and antibiotic resistance

The analysis revealed significant correlations between 16 virulence genes and 9 antibiotic resistance profiles using Pearson's correlation coefficient. Cefepime, ceftazidime, ceftriaxone, imipenem, and meropenem were excluded from the analysis due to the absence of resistant isolates, hence reducing the total number of observations to 21. Among the results, the *cdt*B gene showed a significant positive correlation with resistance to gentamicin ($p = 0.05$), while the *sit*C gene was positively correlated with resistance to piperacillin/tazobactam and nalidixic acid ($p = 0.05$). Based on the analysis, the remaining virulence genes exhibited no significant correlation with antibiotic resistance patterns (Table 3).

Remarkably, *msg*A, *sif*A, and *sop*B were detected in all *Salmonella* serogroup B isolates (Fig. 2). Five strains lacked detectable virulence genes but were still resistant to at least two antibiotics, suggesting alternative mechanisms of resistance. A specific strain from serogroup D (G2-10D) isolated from hand samples exhibited resistance to five antibiotics, classifying it as a MDR strain. Virulence genes were predominantly present in serogroup B isolates, with each isolate containing between 6 and 12 virulence genes. The highest

Table 1 Distribution of *Salmonella* serogroups from different sources of contamination in restaurants.

	Source of isolate				
Serogroup	Kitchen (G1)	Individual (G2)	Dining room (G3)	Total	Proportion (%)
A	0	2	0	2	9.52
B (dT^+)	4	7	0	11	52.38
C1	1	1	0	2	9.52
C2	0	1	0	1	4.76
D	0	2	1	3	14.30
E1	0	1	1	2	9.52
Total	5	14	2	21	100
Proportion (%)	23.81	66.67	9.52	100	

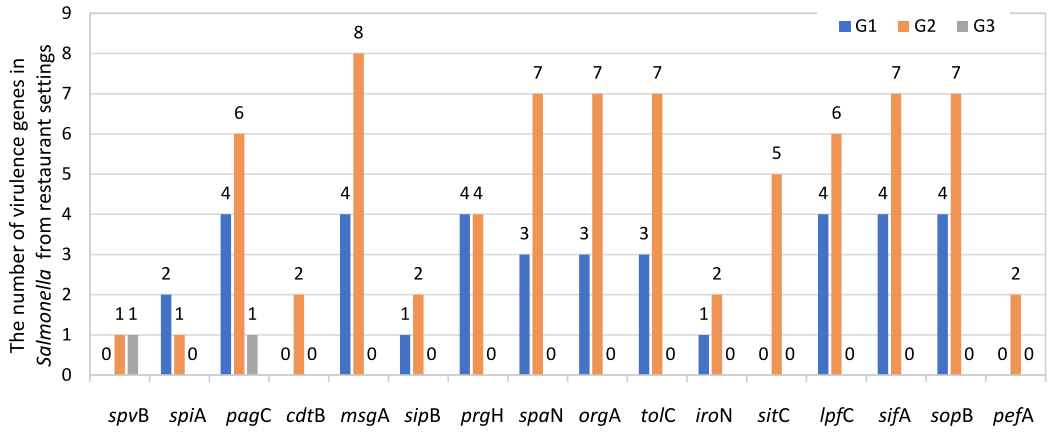

Fig. 1 Distribution of 16 virulence genes in *Salmonella* spp. isolated from restaurants. G1 were from kitchen areas, including the surface of 100 chopping boards and 100 knives; G2 were from individuals, specifically the hands of 75 food handlers and 75 butchers in restaurant settings; and G3 were from the dining areas, encompassing the surfaces of 100 dishes, 100 spoons, and 100 glasses within the restaurants.

Table 2 Antibiotic resistant patterns of *Salmonella* spp. isolated from restaurants.

Isolate name (Source-Isolate no. serogroup)	Antibiotic Resistant Pattern					No. of resistant antibiotic class
G1-2B	AMP	TE	AZM	SXT		4
G1-3B	AZM	TE				2
G1-15B	AZM	TE				2
G1-17-B	AZM	TE	SXT			3
G2-4B	AZM	TE				2
G2-5B	AZM	CTX	C			3
G2-7B	AMP	TE	CIP	NA	PTZ	4
G2-8B	AZM	TE	CIP	NA	PTZ	4
G2-9B	AMP	AZM	NA			3
G2-11B	AMP	TE	AZM	SXT		4
G2-12-B	AMP	TE	AZM	SXT		4
G2-18C2	AZM	TE				2
G2-20A	AMP	AZM				2
G2-24A	AMP	AZM				2
G2-22E1	AMP	AZM				2
G3-19E1	AMP	AZM				2
G2-26C1	–					–
G1-13C1	AZM	TE				2
G2-28D	AZM	TE				2
G3-27D	AZM	CTX				2
G2-10D	AZM	TE	CIP	NA	SXT	4

Table 3 Association of virulence genes with the antibiotic resistant phenotypes.

Gene	PTZ	CTX	AMP	CIP	AZM	NA	C	SXT	TE
*spv*B	-0.105 (0.650)	-0.105 (0.650)	0.375 (0.094)	-0.132 (0.567)	0.105 (0.650)	-0.157 (0.496)	-0.073 (0.755)	-0.181 (0.431)	-0.414 (0.062)
*spi*A	-0.132 (0.567)	-0.132 (0.567)	-0.079 (0.735)	-0.167 (0.470)	0.132 (0.567)	-0.198 (0.390)	-0.091 (0.694)	0.091 (0.694)	0.040 (0.863)
*pag*C	0.309 (0.172)	-0.340 (0.131)	0.248 (0.279)	0.117 (0.614)	0.015 (0.947)	0.220 (0.339)	-0.235 (0.306)	0.085 (0.713)	0.234 (0.308)
*cdt*B	-0.105 (0.650)	0.447 (0.042)*	-0.281 (0.217)	-0.132 (0.567)	0.105 (0.650)	-0.157 (0.496)	0.689 (0.001)**	-0.181 (0.431)	-0.080 (0.732)
*msg*A	0.281 (0.217)	-0.047 (0.840)	-0.028 (0.905)	0.079 (0.735)	-0.281 (0.217)	0.175 (0.448)	0.194 (0.400)	0.258 (0.258)	0.311 (0.169)
*sip*B	-0.132 (0.567)	-0.132 (0.567)	0.196 (0.393)	-0.167 (0.470)	0.132 (0.567)	-0.198 (0.390)	-0.091 (0.694)	0.411 (0.064)	0.040 (0.863)
*prg*H	-0.255 (0.266)	0.080 (0.732)	-0.085 (0.714)	-0.320 (0.157)	0.255 (0.266)	-0.131 (0.572)	0.285 (0.210)	0.252 (0.270)	0.212 (0.357)
*spa*N	0.340 (0.131)	0.015 (0.947)	0.138 (0.552)	0.156 (0.500)	-0.015 (0.947)	0.266 (0.244)	0.235 (0.306)	0.362 (0.106)	0.355 (0.114)
*org*A	0.340 (0.131)	0.015 (0.947)	0.138 (0.552)	0.156 (0.500)	-0.015 (0.947)	0.266 (0.244)	0.235 (0.306)	0.362 (0.106)	0.355 (0.114)
*tol*C	0.340 (0.131)	0.015 (0.947)	0.138 (0.552)	0.156 (0.500)	-0.015 (0.947)	0.266 (0.244)	0.235 (0.306)	0.362 (0.106)	0.355 (0.114)
*iro*N	-0.132 (0.567)	-0.132 (0.567)	0.196 (0.393)	-0.167 (0.470)	0.132 (0.567)	0.149 (0.521)	-0.091 (0.694)	0.411 (0.064)	0.040 (0.863)
*sit*C	0.580 (0.006)**	0.200 (0.386)	-0.032 (0.890)	0.411 (0.064)	-0.200 (0.386)	0.583 (0.006)**	0.400 (0.072)	-0.313 (0.168)	-0.022 (0.925)
*lpf*C	0.340 (0.131)	0.015 (0.947)	0.138 (0.552)	0.156 (0.500)	-0.015 (0.947)	0.266 (0.244)	0.235 (0.306)	0.362 (0.106)	0.355 (0.114)
*sif*A	0.309 (0.172)	-0.015 (0.947)	0.055 (0.813)	0.117 (0.614)	0.015 (0.947)	0.220 (0.339)	0.213 (0.353)	0.309 (0.173)	0.430 (0.052)
*sop*B	0.309 (0.172)	-0.015 (0.947)	0.055 (0.813)	0.117 (0.614)	0.015 (0.947)	0.220 (0.339)	0.213 (0.353)	0.309 (0.173)	0.430 (0.052)
*pef*A	-0.105 (0.650)	-0.105 (0.650)	0.375 (0.094)	-0.132 (0.567)	0.105 (0.650)	-0.157 (0.496)	-0.073 (0.755)	-0.181 (0.431)	-0.414 (0.062)

The numbers in parentheses represent the *p*-value of Pearson correlation statistics.
* Correlation is significant at the 0.05 level (2-tailed) from the Pearson correlation statistics.
** Correlation is significant at the 0.01 level (2-tailed) from the Pearson correlation statistics.

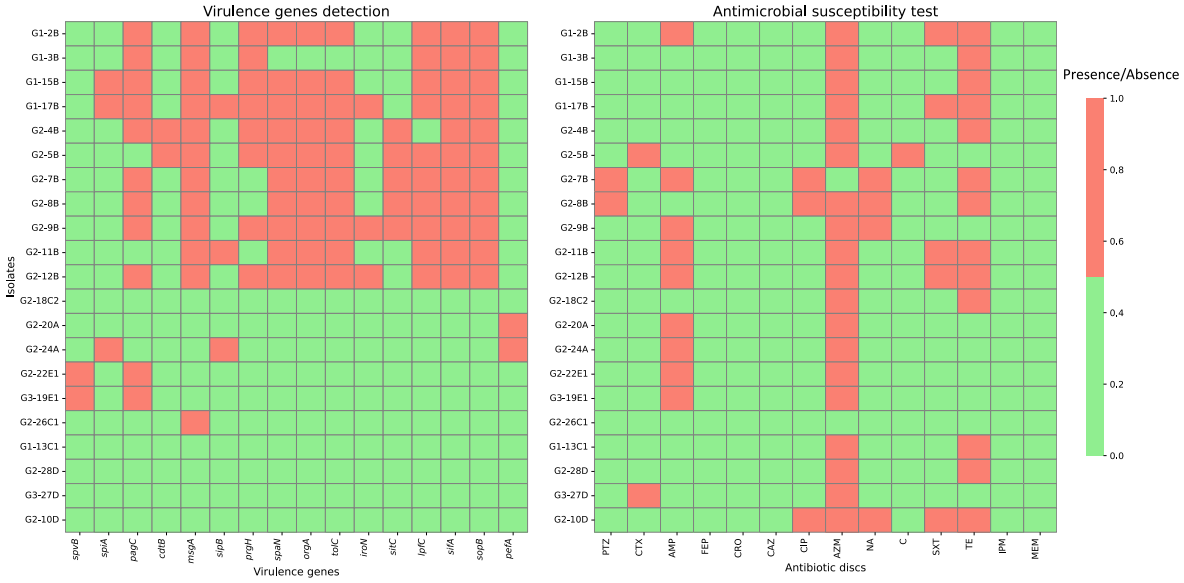

Fig. 2 Sixteen virulence genes (a) and antimicrobial susceptibility testing (b) in *Salmonella* serogroups A to E isolated from three different sources (G1, G2, and G3) in restaurants. Green indicates the absence of virulence genes or an antibiotic-sensitive strain; orange indicates the presence of virulence genes or an antibiotic-resistant strain.

frequency of these genes was observed in kitchen samples, with some isolates harboring up to 12 virulence genes. In contrast, no virulence genes were detected in the three *Salmonella* serogroup D isolates, despite their resistance to five antibiotics across four classes, which also classified them as MDR.

DISCUSSION

The detection of *Salmonella* spp. on food contact surfaces such as hands, knives, chopping boards, and spoons in restaurants is of paramount importance. A total of 650 samples were collected from November and December 2021 from a local restaurant in northern Thailand, divided into three groups: G1 (kitchen), G2 (individuals), and G3 (dining room). All samples were analyzed for *Salmonella* spp., and 21 isolates were identified. These isolates were classified into *S.* Paratyphi B (52.38%), *S.* Typhi (14.30%), *S.* Paratyphi A, serogroup C1, and E1 (each 9.52%), and C2 (4.76%). The detection of *S.* Typhi on the hands of asymptomatic food handlers in restaurant settings represents a significant public health concern. Moreover,

the presence of *E. coli* carrying the *mcr-1* gene, which confers resistance to colistin, together with *Staphylococcus aureus* isolated from the hands of restaurant workers, underscores the potential risk of transmitting both pathogenic and antimicrobial-resistant bacteria through food handling practices. Collectively, these findings highlight critical challenges for public health and emphasize the urgent need for strict hygiene measures, regular monitoring, and effective surveillance in food service establishments [24, 25]. *S.* Typhi is the causative agent of typhoid fever, a potentially life-threatening systemic infection that requires prompt medical intervention. Its presence in food service settings should therefore trigger immediate public health interventions, including health screenings, effective enforcement of hand hygiene, and the education of food handlers to prevent the spread of this highly virulent pathogen. Notably, *S.* Paratyphi B exhibited a high number of virulence genes, with up to 12 virulence genes detected per isolate. These genes are primarily associated with cell invasion, critical for the pathogenicity of *Salmonella*. Other *Salmonella* groups were found to have fewer virulence genes, consistent with previous studies indicating variability in virulence gene numbers across different *Salmonella* serogroups [26, 27]. The detection of up to 12 virulence genes in some isolates from the kitchen environment suggests a high pathogenic potential, especially for strains associated with food contact surfaces. Specifically, three virulence genes: *msg*A, *sop*B, and *sif* A were consistently found in the *Salmonella* group B isolates. In this study, *msg*A was present in 57% of *Salmonella* isolates. In another study, it was found in over 92.3% of all the poultry isolates analyzed [16]. This virulence gene is associated with survival within the host cell. The presence of these three virulence genes across multiple isolates is significant, as they play critical roles in cell invasion and survival within host cells, thereby enhancing the virulence of these strains. The variation in the number of virulence genes among different *Salmonella* serogroups underscores the diversity in pathogenic potential among these strains. Each virulence gene contributes uniquely to the bacterium's pathogenic potential, highlighting the complexity of *Salmonella* pathogenesis. The presence of multiple virulence genes in *Salmonella* isolates, particularly those from the kitchen, suggests an increased potential for pathogenicity and the ability to cause severe infections [14]. The relationship between virulence genes and antibiotic resistance in *Salmonella* is complex and not always straightforward. Some studies suggest a potential trade-off, in which increased resistance may lead to a decrease in virulence. Conversely, other findings demonstrate that virulence and resistance genes can coexist and synergistically enhance each other's effects. Furthermore, the specific virulence genes involved, along with the types of antibiotic resistance, may influence the observed correlations [28, 29]. The observed positive associations between certain virulence genes and antimicrobial resistance in this study underscore the need for further investigation into the molecular mechanisms underlying these interactions [29].

Additionally, the study assessed antibiotic resistance patterns using the disc diffusion method, revealing that 43% of the *Salmonella* isolates were classified as MDR. Among these, 78% were isolated from the hands of restaurant workers. The high incidence of MDR strains is particularly concerning, as it complicates treatment options and poses a significant public health threat. This finding aligns with global trends of increasing antimicrobial resistance in *Salmonella* spp., necessitating vigilant monitoring and effective antimicrobial stewardship [13, 17, 30].

The high rate of MDR among *Salmonella* isolates, especially those isolated from hand samples, is alarming. This emphasizes the critical importance of proper hygiene practices and the prudent use of antibiotics in food production and preparation settings [31].

To mitigate the risk of *Salmonella* contamination in restaurants, it is crucial to implement comprehensive food safety measures, including regular hand hygiene, proper cleaning and sanitization of kitchen equipment, and the implementation of Hazard Analysis and Critical Control Points (HACCP) systems [32]. The presence of MDR strains, especially *S.* Typhi in the restaurant environment, complicates treatment options and increases the risk of severe foodborne illness outbreaks. Therefore, ongoing surveillance and antimicrobial resistance monitoring programs are necessary to track the emergence and spread of MDR *Salmonella* strains.

CONCLUSION

This study demonstrated the presence of *Salmonella* spp. on food contact surfaces and among food handlers in restaurant settings, with several isolates carrying multiple virulence genes and exhibiting multidrug resistance. Critical contamination points included the hands of restaurant workers as well as knives, cutting boards, and serving dishes, all of which can act as vehicles for transmission. The coexistence of virulence determinants and antimicrobial resistance highlights the potential for severe disease outbreaks and complicates treatment options. These findings emphasize the urgent need for strict hygiene enforcement, routine monitoring of food contact articles, and the implementation of HACCP systems to reduce contamination risks. Prudent antibiotic use and effective stewardship programs are also essential to curb the spread of multidrug-resistant *Salmonella*. Further research is warranted to clarify the relationship between virulence and resistance genes and to explore the role of asymptomatic carriers, particularly food handlers, in sustaining the transmission of *Salmonella* in restaurant settings.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2025.089.

Acknowledgements: The authors thank Olalekan Isreal Aikulola, Faculty of Medical Science, Naresuan University, Thailand, for editing of this manuscript. This work was funded by a grant from the National Science, Research and Innovation Fund (NRSF) (2021: R2564B028). This study was approved by the Naresuan University Institutional Review Board (NU IRB).

REFERENCES

1. Lamichhane B, Mawad AMM, Saleh M, Kelley WG, Harrington PJ II, Lovestad CW, Amezcua J, Sarhan MM, et al (2024) Salmonellosis: An overview of epidemiology, pathogenesis, and innovative approaches to mitigate the antimicrobial resistant infections. *Antibiotics* **13**, 76.
2. Popa GL, Papa MI (2021) *Salmonella* spp. infection: A continuous threat worldwide. *Germs* **11**, 88–96.
3. Atwill ER, Jeamsripong S (2021) Bacterial diversity and potential risk factors associated with *Salmonella* contamination of seafood products sold in retail markets in Bangkok, Thailand. *PeerJ* **24**, 9:e12694.
4. Vidayanti IN, Sukon P, Khaengair S, Pulsrikarn C, Angkittitrakul S (2020) Prevalence and antimicrobial resistance of *Salmonella* spp. isolated from chicken meat in upper northeastern Thailand. *Vet Integr Sci* **19**, 121–131.
5. Vo THN, Direksin K, Rattanadilok-Na-Phuket N, Nutravong T, Kerdsin A (2022) Multidrug resistance and serotype distribution of *Salmonella enterica* isolated from homemade recipe fermented ground pork (Nham) in Northeastern Thailand. *Trends Sci* **19**, 1720.
6. Phetsindechakul N, Arkaranupappong U, Chaiyanuwattiwong S, Sehawong P, Keawluanthong W (2022) An outbreak investigation of *Salmonella* food poisoning in a primary school of Mueang district, Nakhon Sawan province, July 2020. *Dis Control J* **48**, 448–457.
7. Tao J, Huang X, Nzekoue FK, Nguefang ML, Zhou X, Zhang Z, Sagratini G, Caprioli G, et al (2024) The relationship between biogenic amines and the growth of spoilage related microorganisms in sliced cooked ham stored under different packaging. *ScienceAsia* **50**, ID 2024044.
8. Soares VM, Pereira JG, Viana C, Izidoro TB, Bersot Ldos S, Pinto JP (2012) Transfer of *Salmonella* Enteritidis to four types of surfaces after cleaning procedures and cross-contamination to tomatoes. *Food Microbiol* **30**, 453–456.
9. Carrasco E, Morales-Rueda A, García-Gimeno RM (2012) Cross-contamination and recontamination by *Salmonella* in foods: A review. *Food Res Int* **45**, 545–556.
10. Raza J, Asmat TM, Mustafa MZ, Ishtiaq H, Mumtaz K, Jalees MM, Samad A, Shah A, et al (2021) Contamination of ready-to-eat street food in Pakistan with *Salmonella* spp.: Implications for consumers and food safety. *Int J Infect Dis* **106**, 123–127.
11. Soubeiga AP, Kpoda DS, Compaoré MKA, Somda-Belemlougri A, Kaseko N, Rouamba SS, Ouedraogo S, Traoré R, et al (2022) Molecular characterization and the antimicrobial resistance profile of *Salmonella* spp. isolated from ready-to-eat foods in Ouagadougou, Burkina Faso. *Int J Microbiol* **9**, 9640828.
12. Alenazy R (2022) Antibiotic resistance in *Salmonella*: Targeting multidrug resistance by understanding efflux pumps, regulators and the inhibitors. *J King Saud Univ Sci* **34**, 102275.
13. Zakir M, Khan M, Umar MI, Murtaza G, Ashraf M, Shamim S (2021) Emerging trends of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Salmonella* Typhi in a tertiary care hospital of Lahore, Pakistan. *Microorganisms* **9**, 2484.
14. Gal-Mor O, Boyle EC, Grassl GA (2014) Same species, different diseases: how and why typhoidal and non-typhoidal *Salmonella enterica serovars* differ. *Front Microbial* **5**, 391.
15. Nikiema MEM, Kakou-Ngazoa S, Ky/Ba A, Sylla A, Bako E, Addablah AYA, Ouoba JB, Sampo E, et al (2021) Characterization of virulence factors of Salmonella isolated from human stools and street food in urban areas of Burkina Faso. *BMC Microbiol* **21**, 338.
16. Lozano-Villegas KJ, Herrera-Sánchez MP, Beltrán-Martínez MA, Cárdenas-Moscoso S, Rondón-Barragán IS (2023) Molecular detection of virulence factors in *Salmonella serovars* isolated from poultry and human samples. *Vet Med Int* **2**, 1875253.
17. Zishiri OT, Mkhize N, Mukaratirwa S (2016) Prevalence of virulence and antimicrobial resistance genes in *Salmonella* spp. isolated from commercial chickens and human clinical isolates from South Africa and Brazil. *Onderstepoort J Vet Res* **83**, 1–11.
18. Levy H, Diallo S, Tennant SM, Livio S, Sow SO, Tapia M, Fields PI, Mikoleit M, et al (2008) PCR method to identify *Salmonella enterica serovars* Typhi, Paratyphi A, and Paratyphi B among *Salmonella* isolates from the blood of patients with clinical enteric fever. *J Clin Microbiol* **46**, 1861–1866.
19. Lim BK, Thong KL. (2009) Application of PCR-based serogrouping of selected *Salmonella* serotypes in Malaysia. *J Infect Dev Ctries* **3**, 420–428.
20. Andrews WH, Wang H, Jacobson A, Ge B, Zhang G, Hammack T (2023) *BAM Chapter 5*: *Salmonella*. US Food & Drug Administration. Available at: https://www.fda.gov/food/laboratory-methods-food/bam-chapter-5-salmonella.
21. Herrera-León S, Ramiro R, Arroyo M, Díez R, Usera MA, Echeita MA (2007) Blind comparison of traditional serotyping with three multiplex PCRs for the identification of *Salmonella* serotypes. *Res Microbiol* **158**, 122–127.
22. Skyberg JA, Logue CM, Nolan LK (2006) Virulence genotyping of *Salmonella* spp. with multiplex PCR. *Avian Dis* **50**, 77–81.
23. CLSI (2025) *Performance Standards for Antimicrobial Susceptibility Testing*, 35th edn, Supplement M100, Clinical and Laboratory Standards Institute (CLSI), Wayne, PA.
24. Thummeepak R, Wongwigkarn J, Kucharoenphaibul S, Sitthisak S, Cheewapat R, Khongfak S, Tasanapak K (2025) Genomic characterization of *mcr-1*-carrying *E. coli* isolated from food handlers. *Trends Sci* **22**, 10562.
25. Tasanapak K, Kucharoenphaibul S, Wongwigkarn J, Sitthisak S, Thummeepak R, Chaibenjawong P, Chatdumrong W, Nimanussornkul K (2023) Prevalence and viru-

lence genes of *Staphylococcus aureus* from food contact surfaces in Thai restaurants. *PeerJ* **14**, 11:e15824.
26. Mokgophi TM, Gcebe N, Fasina F, Adesiyun AA (2024) Molecular characterization of virulence and resistance genes in *Salmonella* strains isolated from chickens sold at the informal chicken market in Gauteng Province, South Africa. *J Food Saf* **44**, e13110.
27. Kumar N, Mohan K, Georges K, Dziva F, Adesiyun AA (2021) Occurrence of virulence and resistance genes in *Salmonella* in cloacae of slaughtered chickens and ducks at pluck shops in Trinidad. *J Food Prot* **84**, 39–46.
28. Beceiro A, Tomás M, Bou G (2013) Antimicrobial resistance and virulence: A successful or deleterious association in the bacterial World? *Clin Microbiol Rev* **26**, 185–230.
29. Yue M, Li X, Liu D, Hu X (2020) Serotypes, antibiotic resistance, and virulence genes of *Salmonella* in children with diarrhea. *J Clin Lab Anal* **34**, e23525.
30. Oueslati W, Rjeibi MR, Benyedem H, Mamlouk A, Souissi F, Selmi R, Ettriqui A (2021) Prevalence, risk factors, antimicrobial resistance and molecular characterization of *Salmonella* in Northeast Tunisia Broiler Flocks. *Vet Sci* **9**, 12.
31. Founou LL, Founou RC, Essack SY (2016) Antibiotic resistance in the food chain: A developing country-perspective. *Front microbiol* **7**, 1881.
32. FDA (2022) *FDA Food Code*. US Public Health Service, US Department of Health and Human Services, USA.

Appendix A. Supplementary data

Table S1 List of primers used for *Salmonella* typing: O serogroup, H typing, and dT-fermentation.

Target	Primer	Sequence (5′ to 3′)	PCR product size (bp)	Ta (°C)	Ref.
B group	*rfb*JF *rfb*JR	CCA GCA CCA GTT CCA ACT TGA TAC GGC TTC CGG CTT TAT TGG TAA GCA	663	50	[19]
D group	*tyv*F *tyv*R	GAG GAA GGG AAA TGA AGC TTT T TAG CAA ACT GTC TCC CAC CAT AC	615	50	[19]
Vi strains	*vi*F *vi*R	GTT ATT TCA GCA TAA GGA G CTT CCA TAC CAC TTT CCG	439	50	[19]
A&D group	*prt*F *prt*R	CTT GCT ATG GAA GAC ATA ACG AAC C CGT CTC CAT CAA AAG CTC CAT AGA	258	50	[19]
C1 group	*wzx*C1F *wzx*C1R	CAG TAG TCC GTA AAA TAC AGG GTG G GGG GCT ATA AAT ACT GTG TTA AAT TCC	483	50	[19, 21]
E group	*wzx*E1F *wzx*E1R	TAA AGT ATA TGG TGC TGA TTT AAC C GTT AAA ATG ACA GAT TGA GCA GAG	345	50	
C2 group	*wzx*C2F *wzx*C2R	ACT GAA GGT GGT ATT TCA TGG G AAG ACA TCC CTA ACT GCC CTG C	153	58	[19, 21]
H:a, H:b, H:d	H-F	ACT CAG GCT TCC CGT AAC GC			[19]
H:a	Ha-R	GAG GCC AGC ACC ATC AAG TGC	423	55	
H:b	Hb-R	GCT TCA TAC AGA CCA TCT TTA GTT G	551	55	
H:d	Hd-R	GGC TAG TAT TGT CCT TAT CGG	763 (d) or 502 (j)	55	
dT-fermentation dT	dT-F dT-R	GTA AGG GTA ATG GGT TCC CAC ATT ATT CGC TCA ATG GAG	289	60	[19]
Internal control *ori*C	P1 P2	TTA TTA GGA TCG CGC CAG GC AAA GAA TAA CCG TTG TTC AC	163		[19]

Table S2 List of primers used for virulence gene detection.

Gene	Primer	Gene location	Pathogenesis	Sequence (5′ to 3′)	PCR product size (bp)	Ref.
*spv*B	spvBF spvBR	Plasmid	Bacterial invasion	CTA TCA GCC CCG CAC GGA GAG CAG TTT TTA GGA GGA GGC GGT GGC GGT GGC ATC ATA	717	[22]
*pef*A	pefAF pefAR	Plasmid	Fimbrial	GCG CCG CTC AGC CGA ACC AG GCA GCA GAA GCC CAG GAA ACA GTG	157	[22]
*cdt*B	cdtBF cdtBR	Genome	Toxin	ACA ACT GTC GCA TCT CGC CCC GTC ATT CAA TTT GCG TGG GTT CTG TAG GTG CGA GT	268	[22]
*tol*C	tolCF tolCR	Genome	Survival inside cells	TAC CCA GGC GCA AAA AGA GGC TAT C CCG CGT TAT CCA GGT TGT TGC	161	[22]
*iro*N	iroNF iroNR	Genome	Iron acquisition	ACT GGC ACG GCT CGC TGT CGC TCT AT CGC TTT ACC GCC GTT CTG CCA CTG C	1205	[22]
*pag*C	pagCF pagCR	PAI	Survival inside cells	CGC CTT TTC CGT GGG GTA TGC GAA GCC GTT TAT TTT TGT AGA GGA GAT GTT	454	[22]
*sip*B	sipBF sipBR	PAI	TTSS*	GGA CGC CGC CCG GGA AAA ACT CTC ACA CTC CCG TCG CCG CCT TCA CAA	875	[22]
*spi*A	spiAF spiAR	PAI	TTSS	CCA GGG GTC GTT AGT GTA TTG CGT GAG ATG CGC GTA ACA AAG AAC CCG TAG TGA TGG ATT	550	[22]
*msg*A	msgAF msgAR	PAI	Survival inside cells	GCC AGG CGC ACG CGA AAT CAT CC GCG ACC AGC CAC ATA TCA GCC TCT TCA AAC	189	[22]
*prg*H	prgHF prgHR	PAI SPI-1	TTSS	GCC CGA GCA GCC TGA GAA GTT AGA AA TGA AAT GAG CGC CCC TTG AGC CAG TC	756	[22]
*spa*N	spaNF spaNR	PAI SPI-1	TTSS	AAA AGC CGT GGA ATC CGT TAG TGA AGT CAG CGC TGG GGA TTA CCG TTT TG	504	[22]
*org*A	orgAF orgAR	PAI SPI-1	TTSS	TTT TTG GCA ATG CAT CAG GGA ACA GGC GAA AGC GGG GAC GGT ATT	255	[22]
*sit*C	sitCF sitCR	PAI	Iron acquisition	CAG TAT ATG CTC AAC GCG ATG TGG GTC TCC CGG GGC GAA AAT AAA GGC TGT GAT GAA C	768	[22]
*lpf*C	lpfCF lpfCR	PAI	Fimbrial	GCC CCG CCT GAA GCC TGT GTT GC AGG TCG CCG CTG TTT GAG GTT GGA TA	641	[22]
*sif*A	sifAF sifAR	PAI	TTSS Invasion	TTT GCC GAA CGC GCC CCC ACA CG GTT GCC TTT TCT TGC GCT TTC CAC CCA TCT	449	[22]
*sop*B	sopBF sopBR	PAI	Invasion TTSS	CGG ACC GGC CAG CAA CAA AAC AAG AAG AA TAG TGA TGC CCG TTA TGC GTG AGT GTA TT	220	[22]

* TTSS: Type three secretion systems.