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Research article

Prevalence and antimicrobial resistance of *Salmonella* spp. isolated from chicken meat in upper northeastern Thailand

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Abstract

Foodborne disease associated with *Salmonella* spp. occurs in several parts of the world, including Thailand. The aim of the present study was to determine the prevalence, antimicrobial resistance, and serovars of *Salmonella* spp. isolated from chicken meat in upper northeastern Thailand. A total of 326 swab samples of fresh chicken meat were collected from wet markets in Khon Kaen, U-don Thani, Nong Khai, Loei, Kalasin, Maha Sarakham, and Bueng Kan provinces, northeastern Thailand, between August and November 2019. All samples were analyzed for *Salmonella* spp., which were isolated and identified using the ISO 6579:2002/AMD 1:2007 method. The isolates were tested for antimicrobial susceptibility using the Kirby-Bauer method, and the serovars of isolates positive for antimicrobial resistance were identified. The overall prevalence of *Salmonella* spp. isolated from chicken meat was 36.2% (118/326), and prevalence in each province was as follows: U-don Thani, 70.9%; Bueng Kan, 66.67%; Khon Kaen, 45.9%; Kalasin, 31.25%; Nong Khai, 29.8%; Maha Sarakham, 26.42% and Loei, 12.5%. Resistance was highest for nalidixic acid at 31%, followed by ampicillin (24%), tetracycline (19%), sulfamethoxazole trimethoprim (8%), norfloxacin (5%), ciprofloxacin (4%), amoxicillin (4%), and chloramphenicol (1%). However, all isolates were susceptible to ceftazidime. Twenty seven serovars were detected, with the serovars Corvallis, Singapore, Kentucky and Agona being the most common. *Salmonella* spp. were detected in a large percentage of the swab samples of chicken meat in every city, indicating a high level of contamination of chicken meat. Given the high resistance of *Salmonella* strains to various antimicrobial, it may be beneficial to find other drugs for *Salmonellosis* treatment and to use antimicrobials more wisely.

Keywords: Antimicrobial resistance, Chicken meat, *Salmonella* spp., Upper northeastern Thailand

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INTRODUCTION

Salmonella spp. are foodborne pathogens of the Enterobacteriaceae family. The guts of humans and other animals are the main habitats of these bacteria (Quinn et al., 2011). Foodborne disease associated with non-typhoidal *Salmonella* spp. (NTS) occurs in several parts of the world, including Thailand. Salmonellosis has several clinical signs, such as gastroenteritis, nausea, vomiting, abdominal pain and lead to bacteremia. The bacteria are readily transmitted among humans, animals and environments (Matias et al., 2010; Castro Rosas, 2012; Hur et al., 2012). Infected individuals may carry bacteria asymptomatically.

Antimicrobial-resistant *Salmonella* spp. are of increasing concern in the poultry meat industry. Several isolates obtained from wet markets have been reported resistant to numerous antimicrobials. Although salmonellosis in humans is self-limiting, generally allowing quick recovery without antimicrobial treatment, antimicrobial therapy is necessary for severe cases and for people infected with antimicrobial-resistant *Salmonella* spp. Individuals infected with antimicrobial-resistant strains are more likely to feel pain, experience prolonged illness, have an increased severity of illness, have higher hospitalization costs, and die than those infected with susceptible *Salmonella* spp. (Nair and Johny, 2019; Procura et al., 2019).

It is essential to provide animal food and food products that are free from *Salmonella* spp. in animal production, especially chicken production. The absence of *Salmonella* contamination is the main indicator of food safety (Tortorello, 2003; Matias et al., 2010; Srianta et al., 2009). Contamination with *Salmonella* spp. may occur not only during the slaughtering process but also in the wet market. Wet markets are popular places where food can be purchased at affordable prices, but they are often linked to outbreaks of disease due to their unhygienic conditions. Therefore, determining the prevalence and occurrence of antimicrobial resistance of *Salmonella* spp. in chicken meat from the wet market may provide information that can help control disease transmission in northeastern Thailand.

MATERIALS and METHODS

Sample Collection

Samples from fresh chicken meat were randomly collected from wet markets (n = 326) by swab collection using transport medium (Cary-Blair Medium, Oxoid). Sampling was conducted in Khon Kaen, U-don Thani, Nong Khai, Maha Sarakham, Kalasin, Loei, and Bueng Kan provinces, upper northeastern Thailand, during August-November 2019. All samples were kept at 4°C and sent for analysis at the Veterinary Diagnostic Laboratory, Animal Hospital, Khon Kaen University, Khon Kaen province, Thailand.

The study was reviewed and approved by the Institutional Animal Care and Use Committee of Khon Kaen University, Thailand (IACUC-KKU-No. 71/63).

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Salmonella spp. Isolation and Identification

Following the ISO 6579:2002/AMD 1:2007 procedure, strains of *Salmonella* spp. were isolated and identified. Each swab sample was placed in 9 ml of buffer peptone water (BPW, Oxoid) for the pre-enrichment step. After incubation at 37°C for 18 to 24 h, 3 loopfuls of BPW culture were transferred to modified semisolid Rappaport-Vassiliadis medium (MSRV, Difco). Loops were used to spot the peripheral area on an MSRV plate. The plate was then incubated at 42°C for 18 to 24 h. A loopful of each positive culture was streaked onto xylose lysine deoxycholate agar (XLD agar, Difco), and the cultures were incubated at 37°C for 18 to 24 h.

Potential *Salmonella* spp. colonies on XLD agar were confirmed by biochemical test. Colonies were transferred to triple sugar iron agar (TSI agar, Oxoid) and motility-indole-lysine agar (MIL agar, Difco) and then incubated 37°C for 18 to 24 h. Presumptive colonies were selected from the TSI and MIL media and stored in brain heart infusion broth (BHI broth, Oxoid) plus 20% glycerol as stock cultures at -80°C.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of the strains isolated from chicken meat was performed by the Kirby-Bauer disk diffusion method according to CLSI (2010-2015) and EUCAST (2016) protocols using nine drugs: tetracycline (TET; 30 µg), chloramphenicol (C; 30 µg), sulfamethoxazole trimethoprim (SXT; 25 µg), norfloxacin (NOR; 10 µg), ampicillin (AMP; 10 µg), nalidixic acid (NA; 30 µg), ceftazidime (CAZ; 30 µg), amoxicillin (AMC; 30 µg), and ciprofloxacin (CIP; 5 µg). All antibiotic disks were from Oxoid and had expiry dates in 2022. Escherichia coli ATCC® 25922 was used as a quality-control strain.

Inoculum was prepared before the test. Colonies of *Salmonella* spp. were suspended in 2 ml 0.85% normal saline (NS) and then diluted to match 0.5 McFarland standard (1.5×10^8 CFU/ml). A sterile cotton swab was dipped into the inoculum tube and used to disperse the inoculum uniformly over the surface of Mueller-Hinton agar (MHA, Oxoid). The appropriate antimicrobial disks were placed on the agar surface by using forceps. After the plates were incubated for 18-24 h at 37°C, the diameter of each inhibition zone was measured by using a ruler. The result was interpreted on the basis of zone diameter interpretative standards from the Clinical and Laboratory Standard Institute (CLSI), and each isolate was classified as resistant, intermediate, or susceptible (Clinical and Institute, 2017). Resistance to more than 3 drugs was considered multidrug resistance for the *Salmonella* spp.

Serotyping

Salmonella spp. serovars identification was conducted at Department of Medical Science, Ministry of Public Health, Thailand. The slide agglutination method was carried out with the commercial antisera according to the manufacturer's instructions (S&A Reagents Ltd, Bangkok, Thailand) to detect the presence of O (somatic) and H (flagellar) antigens. The results were interpreted following the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007).

RESULTS

Prevalence and Serovars of *Salmonella* spp.

Out of the 326 swab samples of chicken meat, 118 were positive for the presence of *Salmonella* spp. (36.2%). The prevalence in the studied cities in northeastern Thailand was 70.9% (U-don Thani), 66.67% (Bueng Kan), 45.9% (Khon Kaen), 31.25% (Kalasin), 29.8% (Nong Khai), 26.42% (Maha Sarakham) and 12.5% (Loei), as shown in and Figure 1. Twenty-seven serovars were identified among the 118 *Salmonella* spp. isolates, with *S*. Corvallis (22 isolates; 18.64%), *S*. Singapore (16 isolates; 13.55%), *S*. Kentucky (11 isolates; 9.32%) and *S*. Agona (9 isolates; 7.63%) being the top 4 predominant serovars (Table 2). Moreover, *S*. Aqua was detected for the first time in chicken meat in northeastern Thailand.
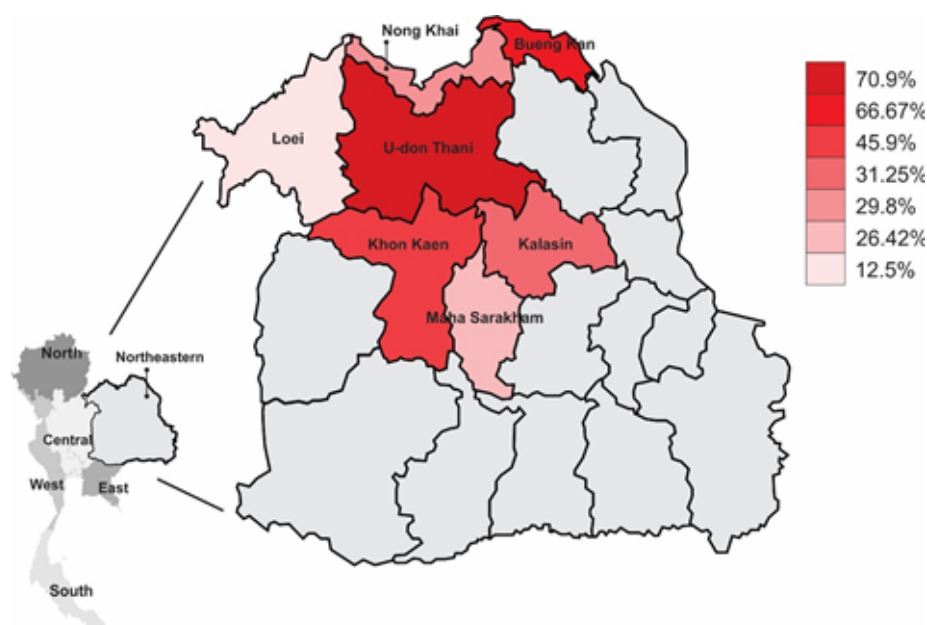

Figure 1 The prevalence of *Salmonella* spp. isolated from chicken meat in different parts of upper northeastern Thailand.

Antimicrobial Resistance among *Salmonella* Serovars.

As presented in Table 1, the percentage resistance of *Salmonella* spp. isolates was highest for nalidixic acid, at 31% (36/118), followed by ampicillin at 24% (28/118), tetracycline at 19% (23/118), sulfamethoxazole trimethoprim at 8% (9/118), norfloxacin at 5% (6/118), ciprofloxacin at 4% (5/118), amoxicillin at 4% (5/118), and chloramphenicol at 1% (1/118). All isolates were susceptible to ceftazidime.

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Table 1 Antimicrobial resistance of *Salmonella* spp. isolated from chicken meat.

Province	AMC	CAZ	CIP	NA	C	TE	SXT	NOR	AMP
Khon Kaen	1	0	2	5	0	8	4	1	7
U-don Thani	0	0	0	14	0	1	1	0	2
Nong Khai	2	0	0	1	1	4	3	1	4
Loei	0	0	0	1	0	2	1	0	3
Maha Sarakham	2	0	2	9	0	7	0	1	7
Kalasin	0	0	1	4	0	4	0	3	5
Bueng Kan	0	0	0	2	0	0	0	0	0
Total	**5/118**	**0**	**5/118**	**36/118**	**1/118**	**23/118**	**9/118**	**6/118**	**28/118**

Note: AMC = amoxicillin, CAZ = ceftazidime, CIP = ciprofloxacin, NA = nalidixic acid, C = chloramphenicol,
TE = tetracycline, SXT = sulfamethoxazole+trimethoprim, NOR = norfloxacin, AMP = ampicillin

Among 118 isolates (27 serovars) of *Salmonella* were antimicrobial susceptibility tested, 52 isolates showed its resistant to at least one antimicrobial drug (44.06%). Sixteen isolates were becoming multi-drug resistant (MDR) (16/118 isolates; 13.55%). As seen in Table 2, the multi-drug resistant isolates were mostly obtained from serovars Rissen (2/3 isolates; 67%), Albany (2/3 isolates; 50%), Agona (4/9 isolates; 44.44%), Kentucky (4/11 isolates; 36%), Stanley (1/5 isolates; 20%), and Corvallis (2/22 isolates; 10%). The fourteen multi-drug resistant isolates shown various pattern as follows: AMC-NA-C-TET-SXT-AMP, AMP-CIP-NA-TET-NOR-AMP (6R); CIP-NA-TET-NOR-AMP (5R); AMC-TET-SXT-AMP, TET-SXT-NOR-AMP, NA-TET-NOR-AMP, NA-TET-NOR-AMP, CIP-NA-TET-AMP, AMC-NA-TET-AMP (4R); and NA-TET-AMP, NA-TET-SXT, TET-SXT-AMP, AMC-NA-AMP, TET-SXT-AMP (3R).

DISCUSSION

An important group of foodborne pathogens in humans, *Salmonella* spp. can enter food at many points in the food production chain. Wet markets are one potential point in the contamination process. Understanding the prevalence or contamination rate in northeastern Thailand region can help control disease transmission. The prevalence of *Salmonella* spp. isolated from chicken meat in wet markets of upper northeastern Thailand was 36.2% (118/326). This prevalence was lower than that reported in previous studies in northern Thailand (57%) and Khon Kaen (44%) (Padungtod and Kaneene, 2006; Neunchat et al., 2017). However, the prevalence of our study was higher than that reported in backyard pigs in Chiang Mai (21%) (Anuchatkitcharoen et al., 2020).

Table 2 Antimicrobial resistance pattern distribution among *Salmonella* serovars.

Salmonella serovars (n)	Predominant resistant patterns	No. of Resistant serovars (%)
Agona (9)	AMC-TET-SXT-AMP	1 (11%)
	NA-TET-AMP	1 (11%)
	NA-TET-SXT	1 (11%)
	TET-SXT-AMP	2 (22%)
	TET-AMP	1 (11%)
	TET-SXT	1 (11%)
Albany (4)	AMC-NA-TET-AMP	1 (25%)
	AMC-NA-AMP	1 (25%)
	NA	1 (25%)
Aqua (1)	-	-
Braenderup (2)	NA	2 (100%)
Bredeney (4)	TET-AMP	2 (50%)
Concord (1)	-	-
Corvallis (22)	AMC-NA-C-TET-SXT-AMP	1 (5%)
	TET-SXT-NOR-AMP	1 (5%)
	NA-AMP	1 (5%)
	NA	4 (18%)
Enteritidis (3)	NA-AMP	2 (67%)
	NA	1 (33%)
Farmsen (1)	-	-
Hvittingfoss (4)	-	-
I ser. 4,5,12 : I : - (1)	TET-AMP	1 (100%)
I ser. 6, 8 : - : 1,5 (1)	-	-
Kedougou (1)	-	-
Kentucky (11)	AMP-CIP-NA-TET-NOR-AMP	1 (9%)
	CIP-NA-TET-NOR-AMP	1 (9%)
	NA-TET-NOR-AMP	1 (9%)
	CIP-NA-TET-AMP	1 (9%)
	CIP-NA	1 (9%)
	NA-NOR	1 (9%)
	TET-AMP	1 (9%)
	AMP	1 (9%)
Mbandaka (3)	-	-
Molade (7)	CIP	1 (14%)
Muenster (6)	-	-
Newport (1)	-	-
Poona (1)	-	-
Ramatgan (2)	-	-
Rissen (3)	TET-SXT-AM	2 (67%)
	TET-AMP	1 (33%)
Schwarzengrund (2)	NA-AMP	2 (100%)
Singapore (16)	NA	11 (69%)
Stanley (5)	NA-TET-NOR-AMP	1 (20%)
Typhimurium (3)	TET-AMP	1 (33%)
Uganda (1)	-	-
Weltevreden (3)	-	-

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The potential factors that may affect the prevalence and detection of *Salmonella* spp. in markets include market management, with markets varying in hygiene or sanitation levels; cross-contamination during slaughtering process; sampling procedures and bacterial isolation-identification procedures. Our study samples were obtained from different geographical area than the areas of previous reports. High prevalence of *Salmonella* spp. was found in U-don Thani (70.9%), Bueng Kan (66.67%), Khon Kaen (45.9%), Kalasin (31.25%), Nong Khai (29.8%), Maha Sarakham (26.42%), and Loei (12.5%) (Figure 1). As a result of varying *Salmonella* spp. percentages from chicken meat, it was determined that *Salmonella* spp. survive well in the environment of wet markets (Nidaullah et al., 2017). In tropical countries, defrosted chicken meat is commonly displayed for sale in the open market without temperature regulation, which presents a contamination risk to chicken products (Wahyono and Utami, 2018). Since the absence of *Salmonella* spp. is considered main indicator of food safety in several countries, including Thailand, the prevalence of this bacteria on chicken meat poses an important health risk. The market management system should consider a permit system for product selling.

In our study, we obtained 118 isolates representing twenty-seven serovars (Table 2). The most commonly found serovar was *S*. Corvallis. This finding is in agreement with several previous findings from northern and central Thailand (Minami et al., 2010; Lampang et al., 2014). Moreover, the prevalence of *S*. Corvallis was higher (22 isolates; 18.64%) in the current study than in previous studies, suggesting that this serovar may be becoming a more common contaminant of chicken products. Yang et al. (2020) reported that *S*. Corvallis was a foremost cause of human infection and has been frequently detected in poultry meat in China. *S*. Singapore (16 isolates; 13.55%), *S*. Kentucky (11 isolates; 9.32%), and *S*. Agona (9 isolates; 7.63%) were among the other serovars found in our study. These serovars were consistent with recent reports worldwide in broilers (VT Nair et al., 2018; Zhang et al., 2018; Phongaran et al., 2019). To the best of our knowledge, the present study provides the first report of *S*. Aqua from chicken meat in northeastern Thailand. Although the finding was not common in Thailand, but National Enteric Disease Surveillance through Center for Disease Control and Prevention (2018) reported that in the period of 2006-2016, culture-confirmed *Salmonella* infection in human linked to *S*. Aqua has been found in several part in the United State (US). Regarding to the report, there was no detail information about the main source and the importance of *S*. Aqua in chicken meat production. However, it should be noted that this serovar may becoming further concern in public health. The distribution of *Salmonella* serovars varies with geographical location, poultry production management, and country development.

S. Typhimurium and *S*. Enteritidis are the most common serovars associated with human salmonellosis outbreaks in several countries and are commonly found in chickens (Demirbilek, 2017). Center for Disease Control and Prevention (2020) reported a recent outbreak related to *S*. Enteritidis, with 78 people infected, 23 hospitalizations and no deaths in the US. Therefore, *S*. Enteritidis is an important source of *Salmonella* infection in humans and an increasing public health concern. Interestingly, we also found *S*. Enterica ser. 4,5,12: i: - in a small number of samples. Soyer et al. (2009) revealed that *S*. Enterica ser. 4,5,12: i: - is antigenic serovar similar to *S*. Enterica ser.

Typhimurium but with less expression of phase II flagella antigen and can also be considered a human salmonellosis pathogen.

The *Salmonella* spp. isolates in our study exhibited the highest resistance to NA (31%), followed by AMP (24%), TET (19%), SXT (8%), NOR (5%), CIP (4%), AMC (4%) and C (1%) (Table 1). Among 118 isolates, 52 isolates showed its resistant to at least one antimicrobial drug (44.06%). Sixteen isolates revealed becoming multi-drug resistant (MDR) (16/118 isolates; 13.55%) which belongs to serovars as follows: Rissen (2/3 isolates; 67%), Albany (2/4 isolates; 50%), Agona (5/9 isolates; 44.44%), Kentucky (4/11 isolates; 36%), Stanley (1/5 isolates; 20%), and Corvallis (2/22 isolates; 10%). Those MDR serovars were consistent with a previous study which was conducted in wet markets in Vietnam and China, where *S*. Rissen, *S*. Agona, *S*. Kentucky, *S*. Stanley and *S*. Corvallis were identified as predominant MDR serovars (Hu et al., 2017; Nhung et al., 2018).

The MDR isolates presented fourteen different antimicrobial resistance pattern as follows: AMC-NA-C-TET-SXT-AMP and AMP-CIP-NA-TET-NOR-AMP (6R); CIP-NA-TET-NOR-AMP (5R); AMC-TET-SXT-AMP, TET-SXT-NOR-AMP, NA-TET-NOR-AMP, NA-TET-NOR-AMP, CIP-NA-TET-AMP, and AMC-NA-TET-AMP (4R); NA-TET-AMP, NA-TET-SXT, TET-SXT-AMP, AMC-NA-AMP, and TET-SXT-AMP (3R). *Salmonella* spp. isolates showed resistance to either tetracycline and ampicillin, which is in agreement with the findings of Shah and Korejo (2012). Nalidixic acid, ampicillin and tetracycline have also been identified as the most common antimicrobial agents to which *Salmonella* spp. isolates exhibit resistance in other countries (Yan et al., 2010; Jeon et al., 2019; Sharma et al., 2019). Some authors have reported an increase in *Salmonella* spp. resistance to quinolones, especially nalidixic acid *(Mølbak et al., 2002; Duc et al., 2019)*. The very frequent occurrence of resistance among *Salmonella* spp. to tetracycline is probably the consequence of the common use of tetracycline as a growth promoter. However, this study revealed that all of the *Salmonella* spp. isolates from chicken meat were susceptible to ceftazidime. A similar finding in north Vietnam has been reported (Thai et al., 2012). Third-generation cephalosporin have been the drugs of choice for salmonellosis treatment in humans for several years. Although, the decreased susceptibility of this drug has been reported in many studied, the number were quite small (Yan et al., 2010; Trongjit et al., 2017).

The use of antimicrobials is one of the main sources of antimicrobial resistance in both humans and animals. Antimicrobials agent kill susceptible bacteria and allow resistant strains to multiply and transmit their resistant genes and residues. Broad-spectrum antimicrobial agents increase the selective burden of bacteria and stimulate the emergence of multidrug-resistant pathogens. The results of the present study reveal single or multiple resistance of *Salmonella* spp. isolated from chicken meat. The potential transmission of these resistant isolates to humans is of great concern.

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CONCLUSION

In this study, *Salmonella* spp. were detected in almost all of the swab samples of chicken meat in every city, indicated a high level of contamination of chicken meat in upper northeastern Thailand. Given the high resistance of *Salmonella* strains to various antimicrobials, it may be beneficial to find other drugs for salmonellosis treatment and to use antimicrobials more wisely.

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AUTHORS CONTRIBUTION

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

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