



## Antibiotic Resistance Profiles of Salmonella Isolated From Different Chicken meat Stalls at a wet Market in Thailand

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### Abstract

Salmonella contamination is a common issue found in chicken meat and other meat products. This foodborne pathogen can survive in various sites in wet markets, especially food contact surfaces. Inadequate cleaning can lead to cross-contamination of this foodborne pathogen with a potential of antibiotic resistant ability in food sold at the wet markets. Ninety- four samples collected from chicken meat (36 samples) and food contact surfaces (58 samples) from three chicken meat stalls, Salmonella was detected in 35 samples (37.2%), of which 19 (57.6%), 8 (23.5%) and 8 (29.6%) samples were from stall A, B, and C, respectively. Chicken meat samples from stall A, B and C showed 66.7%, 50%, and 25%, and food contact surface samples from stall A, B, and C showed 52.4%, 9.1% and 33.3% of samples positive for Salmonella spp., respectively. Fifty-six of the selected Salmonella isolates were characterized for antibiotic resistance, most isolates (38 isolates) showed resistance to tetracycline, followed by ampicillin (26 isolates) and none observed resistance to ceftriaxone. Overall, 21 isolates have been observed as multidrug resistance (MDR), represented by 12 resistant profiles.

**Keywords:** Salmonella spp; Chicken meat stalls; Antibiotic resistance; Wet market



## Introduction

Salmonella spp. is a bacterial pathogen that can cause foodborne illness worldwide. Salmonella contamination is commonly found in retail meats, including beef, lamb, and pork (Yang, B., 2010). Especially, chicken meat is one of the most common vehicles for Salmonella transmission and infection in many countries (Li, K., et al. 2016) It can be transmitted into the body by eating food contaminated with Salmonella. Antibiotic resistance is a worldwide serious issue as it is difficult to treat which can then result in severe illness and death. Multidrug resistant (MDR) Salmonella is also a major issue and often associated with food and other sources, leading to about 100,000 illnesses each year (CDC. Center for Disease Control and Prevention, 2018) According to the National Institute of Health, Department of Medical Sciences, the estimated antibiotic resistant infections each year are more than 100,000 people in Thailand (NARMS. National Antimicrobial Resistance Monitoring System)

Salmonella can be often found on chicken carcasses and other processed chicken products [16]. Salmonella can transfer and reside in various sites in wet markets and can then lead to cross-contamination in food during selling through food contact surfaces or equipment (Osaili, T. M., Alaboudi, A. R. and Nesiari, E. A, 2011). Food contact surfaces such as working tables and equipment in retail processing could also be a source of Salmonella (Tadee, P., Boonkhot, P. and Patchanee, P., 2014) The emergence and transmission of MDR Salmonella suggest a serious health issue and particularly when found related to chicken meat and retail environments where meat is processed and sold. It is important for us to conduct this study to determine the distribution of Salmonella in chicken meat stalls at a wet market in Thailand. Salmonella isolates from a wet market were then investigated for antibiotic resistant ability. Data from this study can suggest the association of antibiotic resistance and Salmonella in retail level which can then increase a risk for spreading to consumers in Thailand.

## Objectives of Research

1. to study Antibiotic Resistance Profiles of Salmonella Isolated Form Different Chicken meat Stalls at a wet Market in Thailand

## Research Methodology

### Locations of Chicken Meat Stalls and Sample Collection

Three chicken meat stalls (A, B and C) located at a wet market in the city of Hat Yai, Songkhla Province, Thailand were included in this study. Ninety-four samples from chicken meat (including breast, drumstick and thigh) and food contact surface were collected during March 2016 to December 2016. All samples were collected at the end of a selling day before store closing.

#### 1. Chicken Meat Samples

For a given stall, two sampling visits were conducted for the collection of 12 raw chicken meat samples (breast, drumstick, and thigh). A total of 6 pieces were randomly collected from each stall. All raw chicken meat samples were kept in sterile bags and placed in an ice box (4°C) during the transportation to the laboratory and analysis within 12 h after sampling

##### 1.1. Food Contact Surface Samples

Food contact surface samples were collected during at least two sampling visits. An additional sampling visit (3rd visit) was included to stalls A and C. At least two samples were collected for the same surface area. Food contact surfaces (FCS) swabs were randomly collected from surfaces of buckets, cutting boards, knives, trays, scales, and working table of each stall; 21 samples from stall A, 22 samples from stall B, and 15 samples from stall C. FCS samples were swabbed by using sterile cotton swab. Cotton swabs were placed back in sterile tubes containing 10 ml of buffered peptone water (BPW). All FCS samples were kept in sterile bags and placed on ice in an ice box (4°C) during the transportation to the laboratory for analysis within 12 h after sampling.

##### 1.2. Salmonella Isolation and Confirmation

Detection of *Salmonella* spp. was performed according to a standard protocol of ISO 6579:2002 with slight modifications (ISO 6579. Microbiology of Food and Animal Feeding Stuff– Horizontal method for the detection of *Salmonella* spp. (4th Ed.). (2002) Geneva, Switzerland. Approximately 25 g from chicken meat sample containing 225 ml of Buffered peptone water (BPW). For FCS sample, 90 ml of BPW was added to each collected swab [24]. After stomaching, the homogenate was incubated at 37 °C for 18 h. Aliquot, 0.1 ml and 1 ml were aseptically transferred 10 ml of Rappaport-Varsiliadis broth with soya (RVS) and Muller - Kauffmann

Tetrathionate-novobiocin broth (MKTTn), and incubated at 41.5 °C for 24 h, and 37 °C for 24 h, respectively. A loopful of samples from RVS and MKTTn was cultured onto two selective agars, Xylose Lysine Desoxycholate (XLD) and Hektoen Enteric (HE), and incubated at 37 °C for 24 h. Up to four presumptive colonies from XLD and/or HE were confirmed by biochemical tests, followed by PCR using *invA* gene (678 bp) [9]. DNA templates for PCR were prepared by using the GF-1 Bacterial DNA Extraction kit (Vivantis, CA, USA) following the manufacturer's instructions. Salmonella isolates were kept in 15% glycerol at -80 °C for further analysis.

### 1.3 Identification of Antibiotic Resistance Profile in Salmonella Isolates

Antibiotic resistance profile was screened by a standard agar disk diffusion method on Mueller-Hinton agar plates, as described by the Clinical and Laboratory Standard Institute [2]. Sixty-six Salmonella isolates of which at least two isolates from each positive samples were followed on nine antibiotics (Oxoid, UK): ampicillin (AMP, 10 µg), chloramphenicol (C, 30 µg), streptomycin (S, 10 µg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg), tetracycline (TE, 30 µg), gentamicin (CN, 10 µg), nalidixic acid (NA, 30 µg), ciprofloxacin (CIP, 5 µg) and ceftriaxone (CRO, 30 µg). *Escherichia coli* (ATCC 25922) was used as a control strain. Results were compared to the standard width of each drug under the table zone diameter interpretive standards and scored as sensitive, intermediate, and resistant according to CLSI guidelines (CLSI, 2015). Two independent replicates were performed.

### Research Results

Occurrence of *Salmonella* spp. Found in Chicken Meat Stalls At A Wet Market: Of 94 samples collected from three chicken meat stalls at a wet market, 35 samples (37.2%) were positive for *Salmonella* spp. (Table 1). Differences in *Salmonella*-positive samples were detected among stalls, i.e., 19 (n = 33; 57.6%), 8 (n = 34; 23.5%) and 8 (n = 27; 29.6%) samples in stall A, B and C, respectively.

Chicken meat samples from stall A, B and C showed 66.7%, 50%, and 25%, and food contact surface samples from stall A, B and C showed 52.4%, 9.1% and 33.3% of samples positive for *Salmonella* spp., respectively. Among samples from these three stalls, the most

frequent occurrence of *Salmonella* spp. in chicken meat and FCS samples were observed in stall A, while less occurrence was observed in chicken meat and FCS samples of stall C and B.

**Table 1** Distribution of *Salmonella* spp. in Chicken Meat Stalls At A Wet Market.

Chicken meat stall	Sampling visit	No. of positive / No. of collected samples (%)		
		Chicken meat	Food contact surface	Total
Stall A	1	4/6	5/5	9/11 (81.8)
	2	4/6	5/10	9/16 (56.2)
	3	nc	1/6	1/6 (16.7)
	Total	8/12 (66.7)	11/21 (52.4)	19/33 (57.6)
Stall B	1	5/6	1/10	6/16 (37.5)
	2	1/6	1/12	2/18 (11.1)
	Total	6/12 (50.0)	2/22 (9.1)	8/34 (23.5)
Stall C	1	2/6	4/5	6/11 (54.5)
	2	1/6	1/8	2/14 (14.3)
	3	nc	0/2	0/2 (0)
	Total	3/12 (25.0)	5/15 (33.3)	8/27 (29.6)

### 1. Antibiotic Resistance Profiles

Among 56 *Salmonella* isolates tested, 10 isolates were susceptible to all nine antibiotics whereas other 50 isolates showed resistant to a single antibiotic or multiple antibiotics (up to 6) (Table 2). *Salmonella* isolates from a given stall showed 10, 5, and 9 antibiotic profiles for stall A, B, and C, respectively.

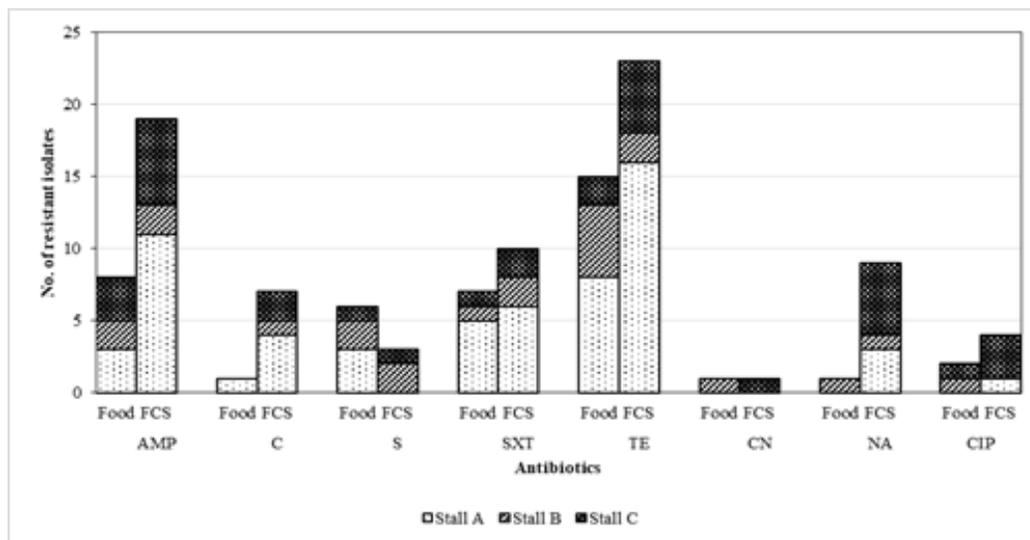
The most common antibiotic resistance of *Salmonella* isolates was tetracycline (40 isolates), followed by ampicillin (28 isolates) (Fig. 1). Only a few isolates (chicken meat isolate from stall B and FCS isolate from stall C) showed resistance to gentamicin. One isolate from chicken meat obtained from stall B and C showed resistance to ciprofloxacin. However, all *Salmonella* isolates were susceptible to ceftriaxone. These profiles provide evidence for multidrug resistance (MDR) as indicated by the resistance of 22 isolates to at least three antibiotics which were presented as 13 profiles (g to s).

Table 2: Distribution of Antibiotic Resistance Profiles of Salmonella Isolates from Chicken Meat and Food Contact Surface Samples.

Antibiotic resistance Profile <sup>a</sup>	No. of isolates						
	Stall A			Stall B		Stall C	
	Visit 1	Visit 2	Visit 3	Visit 1	Visit 2	Visit 1	Visit 2
a (TE only)	6		-	3	-	2	-
b (AMP only)	2	-	-	-	-	-	-
c (AMP-TE)	2	1	-	-	-	-	-
d (AMP-SXT)	-	-	-	-	-	1	-
e (TE-C)	1	-	-	-	-	-	-
f (TE-SXT)	1	1	1	-	-	-	-
g (AMP-CIP-NA)	-	-	-	-	-	-	1
h (AMP-CIP-TE)	-	-	-	-	-	1	-
i (AMP-TE-NA)	-	-	-	-	-	1	-
j (AMP-TE-SXT)	-	-	1	-	-	-	-
k (AMP-CIP-TE-NA)	1	-	-	-	-	2	-
l (AMP-TE-NA-C)	-	-	-	-	-	2	-
m (AMP-TE-SXT-C)	-	2	-	-	-	-	-
n (AMP-NA-SXT-C)	-	2	-	-	-	-	-
o (S-AMP-TE-SXT)	-	3	-	1	-	-	1
p (S-AMP-TE-NA-SXT)	-	-	-	1	-	-	-
q (S-AMP-TE-SXT-C)	-	-	-	1	-	-	-
r (S-CN-AMP-CIP-TE-NA)	-	-	-	-	1	-	-
s (S-CN-AMP-NA-SXT-C)	-	-	-	-	-	1	-
t (Susceptible to all 9 antibiotics)	2	-	-	4	1	3	-
Total	15	13	2	10	2	12	2

<sup>a</sup> Descriptions of antibiotic resistance profiles: (a) TE only; (b) AMP only; (c) AMP-TE; (d) AMP-SXT; (e) TE-C; (f) TE-SXT; (g) AMP-CIP-NA; (h) AMP-CIP-TE; (i) AMP-TE-NA; (j) AMP-TE-SXT; (k) AMP-CIP-TE-NA; (l) AMP-TE-NA-C; (m) AMP-TE-SXT-C; (n) AMP-NA-SXT-C; (o); (p); (q) S-AMP-TE-SXT-C; (r) S-CN-AMP-CIP-TE-NA; (s) S-CN-AMP-NA-SXT-C; (t) Susceptible to all 9 antibiotics.

The most common MDR profile (profile o) found in 5 isolates among chicken meat samples in all stalls, indicated resistance to S-AMP-TE-SXT. For isolates from a given stall, specific MDR profiles were observed. Three MDR profiles (j, m, and n) were specifically found in only FCS isolates from stall A. Two MDR profiles (p and r) were specific to either chicken meat or FCS isolate from stall B only. Five MDR profiles (g, h, i, l, and s) were specific to isolates only from stall C. In addition, profile a (resistance to TE only) was found in isolates from multiple visits to all stalls while profile f (resistance to TE-SXT) showed to be consistently found in chicken meat or FCS isolates from all



three visits to stall A.

**Figure 1.** Antibiotic resistant frequency of Salmonella isolates from different sources. Antibiotics tested: AMP– ampicillin; C– chloramphenicol; S– streptomycin; SXT– trimethoprim-sulfamethoxazole; TE – tetracycline; CN – gentamicin; NA – nalidixic acid; CIP– ciprofloxacin. Sources of isolates: Food (chicken meat), FCS (food contact surfaces).

### Discussion of Research Results

This study investigated the distribution of Salmonella spp. in chicken meat, and food contact surface samples in different chicken meat stalls at a wet market in Hat Yai, Songkhla province, Thailand. Antibiotic resistance profiles were investigated among Salmonella isolates obtained. Overall, samples from three stalls showed 37.2% positive for Salmonella. The most Salmonella-positive samples were chicken meat (25% to 66.7%) and FCS (9.1% to 52.4%) samples

from all three stalls. Typically, Salmonella was found in raw chicken meat, including carcasses, wings, and liver (Lin, D., Yan, M., Lin, S. and Chen, S., 2017) (Goncuoglu, M., Ormanci, F. S. B., Uludag, M. and Cil,) In Thailand, a previous study reported that raw chicken meat from wet markets was frequently contaminated with Salmonella up to 67.5% of samples tested (Lertworapreecha, M., Sutthimusik, S. and Tontikapong, K, 2013)

The distribution of Salmonella in chicken meat at retail markets in some countries in South East Asia has been reported with the rate of 47.5% in Malaysia (Thung, T. Y., Mahyudin, N. A., Basri, D. F., Wan Mohamed Radzi, C. W. J, 2015) and 42.91% in Vietnam (Thai, T. H., Hirai, T., Lan, N. T. and Yamaguchi, R, 2012) However, over 80% of food and environmental samples collected from a wet market in Malaysia has also been reported (Nidaullah, H., Abirami, N., Shamila-Syuhada, A. K., Chuah, L. O., Nurul, H., Tan, T. P., Abidin, F. W. Z. and Rusul, G., 2017) Other regions such as in EU countries, the distribution of Salmonella in fresh broiler meat at retail markets was 7.4% (EFSA and ECDC). In the US, a previous report indicated 8.3 % of broiler carcasses and 22.9% of ground chicken meat were contaminated with Salmonella (USDA, 2015) Findings from this study and a previous study suggest both food and environments of retail operation (wet market) as important sources of Salmonella.

The Occurrence of Salmonella was commonly linked to environmental samples, especially FCS, including cutting board, knives, and other containers, which could indicate improper cleaning. It is possible that inadequate cleaning would link to more Salmonella-positive samples in stall A (in every sampling visit) than other stalls as observed. In all three stalls, Salmonella was often detected on working tables and scales, suggesting important food contact equipment that could be common sources of Salmonella. However, in some study, scales were not a major source of Salmonella contamination (Nidaullah, H., Abirami, N., Shamila-Syuhada, A. K., Chuah, L. O., Nurul, H., Tan, T. P., Abidin, F. W. Z. and Rusul, G., 2017) Generally, Salmonella that survives on chicken carcasses could be transferred onto working tables or scales if carcasses were not washed and/or cleaned properly. An evidence of Salmonella transmission in broiler chicken supply chain was reported by [4] as the same PFGE patterns were found among the majority of isolates from adult broiler, pre- and post- cleaning carcasses, carcasses after chilling, and retail chicken. Salmonella spp. found in retails could link to cross-contamination at various stages, including

slaughtering, processing, and storage at retails (Shafini, A. B., Son, R., Mahyudin, N. A., Rukayadi, Y. and Tuan Z. T. C., 2016).

In this study, antibiotic resistance profiles, including non-MDR and MDR were observed, suggesting widely distributed of *Salmonella* isolates with diverse phenotypic characteristics in chicken meat stalls. This could link to a residue from an inappropriate use of particular antibiotics in poultry farms (Van, T. T. H., Nguyen, H. N. K., Smooker, P. M. and Coloe, P. J, 2012) The presence of MDR isolates in meat may cause serious health issue once the isolates get transferred from food or equipment to humans. The transfer of resistance genes in MDR isolates via horizontal gene transfer to humans may occur and lead to resistant infections (Małka, Ł., Maćkiw, E., Ścieżyńska, H., Pawłowska K. and Popowska, M, 2014) In this study, chicken meats from all three stalls yielded isolates presenting a major MDR profile (profile o; resistance to S-AMP-TE-SXT). This profile is similar to that MDR profile reported among *Salmonella* isolates from retail meats (chicken, beef, and pork) in Hanoi, Vietnam (Nghiem, M. N., Nguyen, V. T., Nguyen, T. T. H., Nguyen, T. D. and Vo, T. T. B, 2017) This finding may suggest a common MDR profile among *Salmonella* associated with poultry meat and poultry retail processing in Thailand and Vietnam. In addition, certain MDR profiles were found to be specific to isolates from each stall, indicating the presence of MDR profile that is source specific in this study. A particular profile was consistently shown in chicken meat or FCS isolates from all three visits to stall A, suggesting a profile that was common among isolates from this stall over time. As in (Chotinun, S., Rojanasthien, S., Unger, F., Tadee, P. and Patchanee, P., 2014) reported 44.3% of *Salmonella* isolates collected from poultry carcasses and their environment such as a bucket, cutting board, knives, etc. Those isolates were resistant to multiple antibiotics and of which 50.6% were MDR.

Overall, *Salmonella* isolates from all stalls and sources showed resistance to tetracycline and ampicillin. Interestingly, high resistance rate to these two and sulfonamides was also found in *Salmonella* from animals and foods of animal origin in Thailand, Malaysia, and Vietnam [26]. The increasing trend of using tetracycline in food animal production could attribute to the occurrence of *Salmonella* resistance to this group of antibiotics (Przzella, C., Ricci, A., DiGiannatale, E., Luzzi, I. and Carattoli, A, 2007 ;Tamang, M. D., Gurung, M., Nam, H. M., Moon, D. C., Jang, G. C., Jung, S. C. and Lim, S. K., 2014) *Salmonella* isolates from retail chicken meats in China also



showed high resistance to tetracycline (Gharieb, R. M., Tartor, Y. H. and Khedr, M. H., 2015).

### Suggestions

Salmonella is commonly found in chicken meat and several environmental surfaces/sites (food contact) at a wet market in Thailand, suggesting potential sources of Salmonella that could be found in meat sold to customers. The results of the research will lead to the development of research into consumption caution that requires research and development on the safety of chicken consumption.

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