# Prevalence and Antimicrobial Resistance of *Salmonella* in Minced Pork from Retail Shops around the University of Phayao, Thailand

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**Abstract**

*Salmonella* is a common pathogen causing food-borne diseases in humans when contaminated food is consumed. As a food commonly consumed in Northern Thailand, raw pork consumption is a common source of contamination. However, there is limited information of the extent and prevalence of *Salmonella* contamination in this region. This current study was a preliminary investigation of the prevalence of *Salmonella* in minced pork from retail shops around the University of Phayao, Thailand, and an analysis of the antimicrobial resistance of the *Salmonella*. A total of 35 minced pork samples were randomly collected from retail shops in the area, and *Salmonella* isolation and identification were performed following ISO 6579. Serogrouping was examined by slide agglutination, and antimicrobial susceptibility testing was performed by the disk diffusion method, based on recommendations of Clinical and Laboratory Standard Institute (CLSI), 2017. Our findings were that a total of 42 *Salmonella* isolates were isolated from 35 samples. The most frequently isolated serogroup was Group C (54.8%), followed by Group B (16.7%) and Group E (14.3%). Antimicrobial susceptibility results revealed that most isolates were resistant to ampicillin (64.3%) and tetracycline (61.9%). Eighteen isolates (43.9%) exhibited multidrug resistant (MDR). The most frequent pattern of MDR was AMP-TE-SXT (19.1%). In summary, 100% of the minced pork samples from food retail outlets around the University of Phayao were contaminated with *Salmonella*, and 43.9% of the isolates were characterized to have a multidrug resistant phenotype.

**Keywords:** *Salmonella*, Minced pork, Serogrouping, Antimicrobial resistance

## Introduction

*Salmonella* is a foodborne pathogen causing illnesses, found worldwide (FAO/WHO, 2016). *Salmonella* frequently resides as part of the intestinal flora of animals, mainly swine and chicken, and can be shed in faeces (Andino & Hanning, 2015; Lertworapreecha, Sutthimusik, & Tontikapong, 2013; Hanson, Kaneene, Padungtod, Hirokawa, & Zeno, 2002). Recent reports suggested that *Salmonella* contamination continues to be a significant problem in food animals (Lertworapreecha et al., 2013; Sanguankiat et al., 2010; Sakdinun, Naksuntorn & Julagivansujarit, n.d.), making contaminated meat unavoidable in the human food supply chain (Berends, Van, Snijders, & Mossel, 1997). The consumption of contaminated meat, and other contaminated food, has resulted in *Salmonella* being prevalent worldwide. The Centers for Disease Control and Prevention, for example, estimates that over 1 million people in the U.S. contract *Salmonella* each year, and that an average of 20,000 hospitalizations and almost 400 deaths occur from *Salmonella* poisoning, according to a 2011 report (http://www.foodborneillness.com/salmonella_food_poisoning/). Previous reports on human infection identified *Salmonella* isolated from patients with diarrhea (Angkititrakul, Chomvarin, Chaita, Kanistanon, & Waethewutajarn, 2005; Sirivan, Arsayuth, Thongsawatwong, & Paugtes, 1996). A high percentage of *Salmonella* contamination has occurred from pork purchased from retail markets (Niyomdecha,

Mungkornkaew, & Samosornsuk, 2016; Lertworapreecha et al., 2013; Sanguankiat et al., 2010; Angkititrakul et al., 2005).

The resistance of *Salmonella* to antimicrobial agents has become a serious public health concern, especially multidrug resistant (MDR) strains, with the severity and duration of infection increasing (Angulo, Nargund, & Chiller, 2004). Therefore, the treatment of infections should be directed towards the strains' antimicrobial susceptibility, which is different in each locality.

Pork is a prominent reservoir of *Salmonella* and is a well-known route of transmission of salmonellosis to humans (Pires, Vieira, Hald, & Cole, 2014; Lertworapreecha et al., 2013; Sanguankiat et al., 2010; Bilhmad, Yoidam, Bhumibhamon, Thongnoon, & Anan, 2007; Angkititrakul et al., 2005; Sirivan et al., 1996; Sakdinun et al., n.d.). Treatment is complicated due to the extent of MDR strains (Niyomdecha et al., 2016; Lertworapreecha et al., 2013).

There were several studies of the prevalence and antimicrobial resistance of *Salmonella* in Thailand (Niyomdecha et al., 2016; Lertworapreecha et al., 2013; Sithigon & Angkititrakul, 2011; Sanguankiat et al., 2010; Bilhmad et al., 2007; Angkititrakul et al., 2005; Sakdinun et al., n.d.), but none were specific to Phayao Province. One area of Phayao with a close-knit population of consumers and food retail shops was the University of Phayao and its immediate environs, which was selected as the study area for the investigation of the prevalence and antimicrobial resistance of *Salmonella* in minced pork collected from the retail shops nearby the University of Phayao.

## Methods and Materials

### Sample collection

Since the University of Phayao is far from the city and clouded community is limited only a main road in front of the University. The retail pork shops were surveyed along the road in front of university of Phayao, with around 2-3 kilometers far from the fence. Thirty-five minced pork samples were collected from all retail shops around the University of Phayao during the period August to October 2017. A number of samples were calculated according to Yamane (1967) for ensure an error level of 0.5% (95% confidence level). Each of the samples was immediately placed in a doubly sterilized plastic bag and stored in an ice box, subsequently transferred to the laboratory within 2 h.

### Isolation and identification

Laboratory testing for *Salmonella* was performed according to ISO 6579 (International Organization for Standardization (ISO) 6579, 2002). Briefly, 25 g of each minced pork sample was suspended in 225 mL BPW pre-enrichment medium (PE) and incubated at 37°C for 18-24 h. An aliquot of 0.1 mL of the PE from the incubated sample was transferred to Rappaport Vassiliadis Soya peptone (RVS, MERCK) broth and incubated at 41.5°C for 18-24 h, while another 1 mL of the PE from the incubated sample was transferred to 9 mL tetrathionate broth (TTB, HIMEDIA) and incubated at 37°C for 18-24 h. After 18-24 h of incubation of the second broth set a loop of each sample was plated on selective and differential medium, xylose lysine deoxycholate agar (XLD, OXOID), and incubated at 37°C for 18-24 h. At least 3 suspected

colonies were chosen to be grown on triple sugar iron medium (TSI, OXOID) and motility indole lysine decarboxylase medium (MIL, Difo) for further identification.

*Salmonella* serogrouping was performed by slide agglutination with O *Salmonella* antisera (S&A reagent, Thailand). *Salmonella* prevalence was calculated by the total number of *Salmonella* positive samples divided by the total number of samples. Additionally, the percentage of MDR and ESBL *Salmonella* were calculated by the total number of antimicrobial resistant isolates divided by the total number of *Salmonella* isolates.

**Antimicrobial Susceptibility Testing**

All *Salmonella* positive isolates were tested for susceptibility to 12 antimicrobial agents (Oxoids, England); ampicillin (10 µg), Amoxicillin-clavulanate (20/10 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), tetracycline (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg) and chloramphenicol (30 µg). Susceptibility testing was performed according to recommendations of the Clinical and Laboratory Standards Institute (CLSI) 2017, with using *Escherichia coli* ATCC 25922 as a quality control. Inhibition zones were measured and interpreted accordingly by CLSI guidelines, and the ESBL production was performed by the disk combination method among the stains resistant to any third cephalosporin agents.

## Results

**Prevalence of *Salmonella***

The prevalence of *Salmonella* in the minced pork samples was 100%, with the most prevalent serogroup being Group C (54.8%) followed by Group B (16.7%), Group E (14.3%), Group G (9.5%), Group I (2.4%) and non-Group A-I (2.4%) (Table 1).

**Table 1** Prevalence of *Salmonella* serogroup from minced pork around University of Phayao

| Serogroup | Number of positive (%) |
|---|---|
| Group B | 7 (16.7) |
| Group C | 23 (54.8) |
| Group E | 6 (14.3) |
| Group G | 4 (9.5) |
| Group I | 1 (2.4) |
| Non Group A-I | 1 (2.4) |
| **Total** | **42 (100)** |

**Antimicrobial resistance of *Salmonella***

Antimicrobial susceptibility testing was performed on all 42 isolates. The results revealed that these *Salmonella* isolate are resistant to ampicillin (64.3%), amoxicillin-clavulanate (2.4%), cefotaxime (7.1%), ceftazidime (4.8%), ceftriaxone (7.1%), cefepime (4.8%), tetracycline (61.9%), nalidixic acid (2.4%), trimethoprim-sulfamethoxazole (28.6%) and chloramphenicol (19.1%). As well, all of the *Salmonella* isolates were still sensitive to ciprofloxacin and norfloxacin (Table 2).

**Table 2** Antimicrobial resistance of *Salmonella* isolated from minced pork around University of Phayao

| Serogroup | Resistance (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AMP | AMC | CTX | CAZ | CRO | FEP | TE | NA | CIP | NOR | SXT | C |
| Group B | 5 (11.9) | 0 | 0 | 0 | 0 | 0 | 6 (14.3) | 0 | 0 | 0 | 0 | 1 (2.4) |
| Group C | 18 (42.9) | 0 | 2 (4.8) | 1 (2.4) | 2 (4.8) | 1 (2.4) | 16 (38.1) | 1 (2.4) | 0 | 0 | 11 (26.2) | 6 (14.3) |
| Group E | 1 (2.4) | 0 | 1 (2.4) | 1 (2.4) | 1 (2.4) | 1 (2.4) | 1 (2.4) | 0 | 0 | 0 | 0 | 1 (2.4) |
| Group G | 2 (4.8) | 1 (2.4) | 0 | 0 | 0 | 0 | 3 (7.1) | 0 | 0 | 0 | 1 (2.4) | 0 |
| Group I | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Non Group A-I | 1 (2.4) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Total** | **27 (64.3)** | **1 (2.4)** | **3 (7.1)** | **2 (4.8)** | **3 (7.1)** | **2 (4.8)** | **26 (61.9)** | **1 (2.4)** | **0** | **0** | **12 (28.6)** | **8 (19.1)** |

**Note:** AMP; ampicillin, AMC; amoxicillin-clavulanate, CTX; cefotaxime, CAZ; ceftazidime, CRO; ceftriaxone, FEP; cefepime, TE; tetracycline, NA; nalidixic acid, CIP; ciprofloxacin, NOR; norfloxacin, SXT; trimethoprim-sulfamethoxazole and C; chloramphenicol

The results also showed the percentage of MDR *Salmonella* was 43.9% (18/42). The common pattern of MDR found in Group C was AMP-TE-SXT (19.1%) and followed by AMP-TE-SXT-C (7.1%). All patterns of MDR are presented in Table 3. In addition, 3 MDR isolates were phenotypic confirmed as ESBL producing *Salmonella*, with the frequency of 7.1%.

**Table 3** Patterns of MDR *Salmonella* in each serogroup isolated from minced pork around University of Phayao

| Serogroup | Number of MDR (%) |
|---|---|
| Group B | |
| AMP TE C | 1 (2.4) |
| Group C | |
| AMP TE C | 1 (2.4) |
| AMP TE SXT | 8 (19.1) |
| AMP TE C SXT | 3 (7.1) |
| AMP TE C CTX CAZ CRO | 1 (2.4)* |
| AMP TE C CTX CAZ CRO FEP NA | 1 (2.4)* |
| Group E | |
| AMP TE C CTX CAZ CRO FEP | 1 (2.4)* |
| Group G | |
| AMP TE SXT | 1 (2.4) |
| AMP TE AMC | 1 (2.4) |
| Group I | 0 |
| Non Group A-I | 0 |
| **Total** | **18 (43.9)** |

**Note**: * ESBL positive

## Discussion

*Salmonella* is a common foodborne pathogen leading to illnesses and death in humans (FAO/WHO, 2016). The course of *Salmonella* infection has increased both in incidence and severity (Lertworapreecha et al, 2013). Consumption of *Salmonella* contaminated food is a significant risk for infection, and it is well-reported that pork is a source of *Salmonella* contamination (Pires et al., 2014; Lertworapreecha et al., 2013; Sanguankiat et al., 2010; Bilhmad et al., 2007; Angkititrakul et al., 2005; Sakdinun et al., n.d.) leading to human Salmonellosis (Sirivan et al., 1996).

An increase in the number of antimicrobially resistant *Salmonella* strains is likely to increase the difficulty in treatment and the mortality rate. Therefore, epidemiological study of the prevalence and antimicrobial susceptibility is an essential part of the process for controlling and surveillance of *Salmonella* outbreaks in the public health system (Lertworapreecha et al, 2013). According to the standards of microbiological quality of food and food containers, Thailand (Bureau of Quality and Safety of Food, 2017), animal meat available for sale and consumption be totally free of *Salmonella* contamination.

This study showed that 100% of the minced pork samples tested were contaminated by *Salmonella*. This high prevalence is consistent with previous studies in Khon Kaen Province (65% sample contamination) (Angkititrakul et al., 2005) and Phatthalung Province (82% sample contamination) (Lertworapreecha et al, 2013).

In our present study, the most frequently isolated serogroup was Group C (54.8%), followed by group B (16.7%), E (14.3%), and G (9.5%), which is again consistent with results from Khon Kaen (Group C (61.5%), B (11.5%), E (19.1%) and G (3.8%)) (Angkititrakul et al., 2005). Antimicrobial susceptibility results revealed that most *Salmonella* isolates in our study were resistant to AMP (64.3%), TE (61.9%),

SXT (28.6%) and C (19.1%) and no resistance to fluoroquinilones (CIP and NOR), which are typically the primary drugs of choice for clinical treatment (Lertworapreecha et al., 2013; Bilhmad et al., 2007; Angkititrakul et al., 2005; Glynn et al., 1998). MDR of *Salmonella* has been evaluated according to the guide lines of resistance to ≥1 agent in ≥3 antimicrobial class (Magiorakos et al., 2012). The most prevalent combination of MDR results was AMP-TE-SXT (19.1%), followed by AMP-TE-C-SXT (7.1%). Interestingly, three of those MDR were ESBL producing strains. Therefore, it is strongly indicated that MDR *Salmonella* in pork is a route of transmission into people (Lertworapreecha et al., 2013). MDR strains can increase virulence and invasiveness of the pathogen, as well as cause higher mortality rates compared to drug-susceptible *Salmonella* strains (Andino & Hanning, 2015). Of concern is the fact that MDR *Salmonella* may remain viable in food animals during processing production even when sub-therapeutic doses of antibiotics are used.

Although *Salmonella* is the normal flora in the gut of pigs, pork sold for human consumption must be 100% free of *Salmonella* contamination, according to the guideline of Bureau of Quality and Safety of Food (2017). This food safety requirement must be compared with the 100% prevalence of *Salmonella* contamination of minced pork in retail shops around University of Phayao, strongly suggesting extremely low food hygiene practices in pork processing and preparation facilities. Previous studies in Thailand showed that *Samonella* contamination could be found in almost every link in the food supply chain, from the farm, in the slaughterhouse, during transportation, and in the retail markets (Niyomdecha et al., 2016; Lertworapreecha et al., 2013; Sithigon & Angkititrakul, 2011; Sanguankiat et al., 2010; Bilhmad et al., 2007; Angkititrakul et al., 2005; Hanson et al., 2002; Sirivan et al., 1996; Sakdinun et al., n.d.). Furthermore, a high prevalence of *Salmonella* contamination in pork were found in several other areas studied (Pires et al., 2014). For example, contamination of pork in slaughterhouses located in the western and southern provinces of Thailand were found in 44.39% of western facilities and 89.85% in southern facilities (Sakdinun et al., n.d.; Bilhmad et al., 2007). The source of contamination in slaughterhouse occurred during cutting process specifically from cutting boards (55%), knives (30%), staff hand (40%), water (19.51%) and worker rectal swab (10.71%) (Sithigon & Angkititrakul, 2011; Sanguankiat et al., 2010). Other studies reported the percentage of *Salmonella* contamination in pork samples from retail markets in many regions of Thailand; Chiang Mai 39.4% (Sanguankiat et al., 2010), Phatthalung 82% (Lertworapreecha et al., 2013), Khon Kaen 65% (Angkititrakul et al., 2005) and Bangkok 82% (Niyomdecha et al., 2016).

*Salmonella* contamination can occur from the earliest stages of the slaughter process indicating that attention should focus on all stages of the pork production chain to reduce contamination and risk of infection of the meat. Unfortunately, contamination during the processing of pork products is unavoidable when slaughter work is conducted routinely and the cutting process is run continuously (Berends et al., 1997). Therefore, strict measures to reduce contamination during the processing, such as personal hygiene and plant sanitation programs, should be applied vigorously. A comprehensive quality safety and assurance scheme, such as Hazard Analysis Critical Control Point (HACCP) (USDA, 1999), which also includes staff educational programs, should help to increase the level of awareness of food hygiene at all stages in the food chain, particularly at the farm and slaughterhouse level (Legnani, Leoni, Berveglieri, Mirolo & Alvaro, 2004; Van der Gaag, Saatkamp, Backus, Beek & Huirne, 2004).

## Conclusion and Suggestion

Our preliminary survey of *Salmonella* contamination found a high level of contamination in the minced pork collected around the University of Phayao, with contamination in 100% of the samples. The most frequently occurring serogroup was serogroup C, with some of the isolates being MDR. ESBL-producing strains were also found. These results must be viewed with great concern. However, the number of samples taken, and the extent of the geographic area of this study, were limited. Our further studies will include the monitoring of the prevalence of, and antimicrobial resistance of, *Salmonella* in a wider area in Northern Thailand. Moreover, both serotyping for *Salmonella* and molecular typing of antimicrobial resistant genes should be considered.

## Acknowledgement

The authors thank the School of Allied Health Sciences, University of Phayao, for support by the provision of laboratory chemicals and facilities, and the students in the Food Microbiology course (382474) for helping in collecting samples, and the isolation, identification and antimicrobial susceptibility testing of *Salmonella*. I would like to thank Ms. Karen Stangl for manuscript correction. Thanks also to Mr Roy I. Morien of the Naresuan University Graduate School for his editing assistance on English expression and grammar.

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