



Antimicrobial Resistance Profiles of *Salmonella* spp. Isolated from Swine Feces in Phayao Province, Thailand

Nitsara Boonkerd^{a*} & Surasak Chaikhiandee^b

^a Microbiology and Parasitology, School of Medical Science, University of Phayao, Phayao, 65000 Thailand

^b Nutrition and Dietetics, School of Medical Science, University of Phayao, Phayao, 65000 Thailand

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Abstract

Antimicrobial resistance in *Salmonella* spp. is a serious issue for food safety. In Thailand, antibiotics are being used more frequently for both prophylaxis and treatment as commercial swine farming expands. Antimicrobial resistance has been generated as a result of the widespread use of antimicrobials in animal agriculture. For this study, we examined the antimicrobial resistance profiles and resistance percentages of *Salmonella* spp., which were isolated from swine fecal samples in Phayao province. The results showed that the overall prevalence of *Salmonella* spp. in the fecal samples in Phayao Province was 49.17%. The highest prevalence of *Salmonella* spp. contamination was found in the piglet fecal samples (70%), followed by sick swine fecal samples (65%) and adult swine fecal samples (40%). The recovered 100 *Salmonella* spp. isolated from adult swine, sick swine and piglet feces were 34, 22 and 44 respectively. After that all isolates were tested for antimicrobial susceptibility. It was found that the highest resistance rate to ampicillin, which were equal to 79.41, 81.82 and 95.45 % in isolates from adult swine, sick swine, and piglets, respectively. *Salmonella* spp. isolates were resistant to ampicillin (87%), trimethoprim-sulfamethoxazole (84%), tetracycline (62%) and chloramphenicol (61%). Interestingly, *Salmonella* spp. isolated from piglets had highest percentage of resistance. *Salmonella* spp. isolated were resistant to ampicillin, trimethoprim-sulfamethoxazole, tetracycline, chloramphenicol, and cefotaxime which were equal to 95.45, 95.45, 90.91, 56.82 and 52.27%, respectively. MDR *Salmonella* was observed among 87 of 100 (87%) isolates. Whereas seventeen different multidrug-resistant profiles were observed. The most frequently found antimicrobial resistance profiles was AMP-SXT-TE-CIP. Furthermore, the probability of resistance to antimicrobial agents has increased. Further description of the associations between resistance and how resistance spreads within farms, are required before effective intervention strategies can be designed to control MDR *Salmonella* in swine.

Introduction

Salmonella spp. is the bacterial pathogen that is ubiquitously found in the human food chain. Previous studies have shown that *Salmonella* is one of the leading foodborne pathogens (Duggan et al., 2010), and plays a significant role for causing human diarrhea in various countries (Pan et al., 2018). Salmonellosis is caused by *Salmonella* contamination in food. In the epidemiology of *Salmonella* spp., food acts as the main source of infection and animal asymptomatic carriers (Denis et al., 2013). Farms are natural reservoir of *Salmonella*, especially poultry and swine (Xu et al., 2020). Pork meat is one of the major foods from an animal which is produced and consumed in Thailand. The swine production consumption has indirectly increased the risk of foodborne zoonoses. *Salmonella* could colonize the digestive tract of swine and excreted in feces and spread into the environment (Jiang et al., 2019). As a result, *Salmonella* feasibly transmitted to humans via the food chain. Whereas transmission of *Salmonella* among swine occurs mainly via the fecal–oral route. The prevalence of infection in swine on the farm that might be triggered by stress factors linked to group housing, transportation and holding pens at the slaughterhouse, as the physiological changes associated with stress could promote in carriers or increase the susceptibility of non-carriers to new infections. Thailand's livestock department in 2020 reported the primary swine-producing area is in the central region of Thailand. In the northern region, there are 1,194,042 swine and 41,931 swine farmers, which is classified as the second largest swine producing area of Thailand. For the northern region, the data on the *Salmonella* contamination in three provinces (Chiang Mai, Chiang Rai, and Lamphun) have been established (Patchanee et al., 2015a, Tadee et al., 2021). However, the data on the prevalence and antimicrobial resistance of *Salmonella* in Phayao Province have not been identified.

Antibiotic-resistant microorganisms are currently a major concern to both human and animal health. Antimicrobial resistance is a serious ongoing global concern when it comes to zoonotic *Salmonella*. This becoming more complicated due to the emergence of the pathogenic strains resistant to many antimicrobial agents simultaneously. These pathogenic strains were called multidrug resistance (MDR). MDR *Salmonella* caused foodborne illness outbreaks through contaminated pork products in 2015, which resulted in severe infection in

humans. *Salmonella* isolates were resistant to multiple antimicrobial agents, including ampicillin, streptomycin, sulfamethoxazole, and tetracycline (CDC, 2015). Antimicrobial agents are used in food animal production to promote growth and to prevent, treat, and control infectious diseases (Sneeringer et al., 2015). Previous research suggested that the amount of antimicrobial agents consumed by swine outweighed the usage of antibiotics for non-therapeutic purposes. Tetracycline and sulfonamides were two antimicrobials that were frequently used in swine production to enhance productivity or as therapeutics. Also, antibiotics were found to be an effective against mortality and morbidity in piglets to diseases (Cromwell, 2002). However, excessive use and over the counter purchase of antimicrobial agents in Thailand is common in both humans and farm animals. The use of antimicrobial agents as a feed additive in farm animals is rarely carried out under veterinarian supervision. This causes a rapid increase in both the animal's resistance to certain bacteria and in the level of antimicrobial residues in animal products. Thus, antibiotic resistance has increasingly emerged and re-emerged as a major threat to public health and economy in various countries. Furthermore, the population dynamics of antibiotic-resistant *Salmonella* spp. varies in swine due to the varying selection pressure exerted by the different antimicrobial agents (Seuberlich et al., 2009). As a consequence, it is crucial to have an improved surveillance system for pathogens with antimicrobial resistance in animal-borne foods. Furthermore, it is important to note the scarcity of evidence on the epidemiology of *Salmonella* infection in the production stages, including piglets, adults, and sick swine. These phases may influence the dynamics of infection. The monitoring of MDR *Salmonella* spp. in animal are essential to effectively control antimicrobial resistance. An improved understanding is essential to evaluate the risk of *Salmonella* contamination in the swine. Furthermore, significant advancements have been achieved in understanding and prediction of antimicrobial resistance of the *Salmonella*. Because of this, we have been strongly encouraged to look into the prevalence, percentage of resistance, and antimicrobial resistance profiles of *Salmonella* spp. found in swine fecal samples in Phayao Province.

Materials and methods

1. Sample collection

During 2020, 120 Fecal samples were collected after swine excreted on the floor. The random samples were collected from a medium swine farm (21-100 swine) in Phayao, a province in Northern Thailand, for *salmonella* isolation and identification. Fecal samples were divided into 3 sample groups. Fecal samples incorporated 80 adult swine fecal samples (aged swine from 10 weeks to 24 weeks old), 20 sick swine fecal samples (swine that were diagnosed with diarrhea) and 20 piglet fecal samples (weaning swine up to 10 weeks old) (Table 1). Fecal samples were collected by cotton and transferred by Cary-Blair transport media for analysis in a laboratory at the School of Medical Science, University of Phayao.

Table 1 Number of swine fecal samples in each group

Group of sample	Number of sample
Adult Swine fecal swab	80
Sick swine fecal swab	20
Piglet fecal swab	20
Total	120

2. *Salmonella* spp. identification

The swine fecal sample swabs were streaked on *Salmonella*-Shigella agar and incubated overnight at 35°C. One to five black colonies were selected. Potential *Salmonella* spp. colonies on *Salmonella*-Shigella agar were confirmed by Gram staining and biochemical test. Colonies were transferred to triple sugar iron agar (TSI), sulfide indole motility medium (SIM) and motility-indole-lysine medium (MIL) and then incubated 37°C for 18 to 24 h for confirmation. Then *Salmonella* spp. isolates were stored in 20% glycerol at -80°C.

3. Antimicrobial susceptibility test

According to the standard operational procedures, antimicrobial susceptibility tests were done on Mueller-Hinton agar using Kirby-Bauer disk diffusion method. Concisely, using a sterile loop, pure colonies were picked from nutrient agar and emulsified in normal saline and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was then adjusted to the optical density of 0.5 McFarland. A sterile cotton swab was then dipped into the suspension and distributed the bacteria suspension evenly over the entire surface of Mueller-Hinton agar. The antimicrobial agents included ampicillin (AMP) 10 µg, ceftazidime (CAZ) 30 µg, cefotaxime (CTX) 30 µg, chloramphenicol

(C) 30 µg, trimethoprim-sulfamethoxazole (SXT) 1.25/23.75 µg, meropenem (MEM) 10 µg, ciprofloxacin (CIP) 5 µg and tetracycline (TET) 30 µg. The plates were then incubated at 37°C for 18 h. The zone of inhibition for each *E. coli* isolate was analyzed according to the standards and the interpretive criteria of CLSI. (Clinical Laboratory Standards Institute, 2017). *Escherichia coli* ATCC 25922, which is a recommended reference strain for antimicrobial susceptibility testing, was used as a control. MDR isolates were defined as resistant to at least one agent in three or more antimicrobial classes.

4. Statistical analysis

Data were displayed as percentages and numbers The prevalence of *Salmonella* spp. was estimated based on the number of positive samples and any associations between groups of swine were determined using the chi-square test for independence. A *p* value of 0.05 was required for statistical significance. Data was analyzed using SPSS Software for Windows, Version 20.0.

Results and discussion

Salmonella spp. is the major cause of foodborne gastrointestinal illnesses in humans (Herikstad et al., 2002). Food-producing swine is an important source of *Salmonella* spp. in food products (Alban et al., 2002). An estimated 23% of all cases of human salmonellosis are related to the consumption of meat (Duggan et al., 2010). Antimicrobial resistance is a great problem of public health. In recent years, a high percentage of antimicrobial-resistant *Salmonella* spp. was frequently observed in all countries. Especially, *Salmonella* spp. showed resistance to tetracycline, sulfonamides/sulfamethoxazole, and ampicillin. Moreover, an increasing number of multidrug-resistant isolates were recovered (EFSA, 2020). There is growing concern about multidrug-resistant (MDR) *Salmonella*, especially the effectiveness of important antimicrobial agents, such as fluoroquinolones and extended-spectrum cephalosporins, which are the drug of choices used for treatment of salmonellosis in human (Crump & Mintz, 2010). Reservoirs of multidrug resistance are found in swine farms in Northern Thailand. They may be affected by antimicrobial usage on the farm. Access to antimicrobial agents as a medicated feed appeared to be an important factor to consider regarding the development of drug resistance in swine farms.

In this study, a total of 120 swine fecal samples (80 adult swine fecal samples, 20 sick swine fecal

samples and 20 piglet fecal samples) were collected in Muang District, Phayao Province. Including 240 black colonies on *Salmonella*-Shigella agar were selected. All suspected colonies of *Salmonella* spp. were confirmed by Gram staining and biochemical analysis. In Gram staining, the morphology of the isolated bacteria was gram negative and rod shape (Fig. 1). For biochemical test, triple sugar iron (TSI) test of the *Salmonella* isolates showed fermentation of glucose and H₂S formation. The urease and indole tests for these isolates were negative. Whereas the motility and lysine decarboxylase were positive (Fig. 2). The overall prevalence of *Salmonella* spp. in the fecal samples in Phayao Province was 49.17% (59/120) which is higher than rates identified in swine farms from the same region, Tadee et al., reported occurrence of 31% (Tadee et al., 2014) and 25% (Tadee et al., 2021). The highest prevalence of *Salmonella* spp. contamination was found in the piglet fecal samples (70%; 14/20), followed by sick swine fecal samples (65%; 13/20) and adult swine fecal samples (40%; 32/80) (Table 2). We found significant differences between groups ($p \leq 0.05$). The overall higher prevalence of shedding was observed when compared to previous studies. Another potential factor influencing was related to shedding which become exacerbated by the stress associated with the transport and lairage making *Salmonella* detection possible (Arguello et al., 2013). The results showed that recovered 100 *Salmonella* spp. isolates from adult swine, sick swine and piglet feces were 34, 22 and 44 respectively (Table 2). According to the study of Vigo et al., (2009), reported that shedding of *Salmonella* spp. to peak during the nursery period and subsequently decrease over time. Besides, stress associated with travel is reported to alter the pathogen release along with a variety of other factors, including environmental contamination and dose-response parameters (Simons et al., 2016).



Fig. 1 Gram staining morphological observation of *Salmonella* spp. (100X)

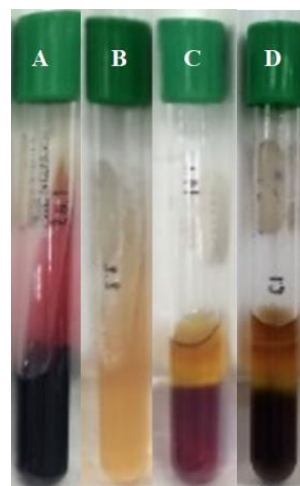


Fig. 2 Biochemical test for *Salmonella* spp. For TSI agar, Fermentation of glucose and hydrogen sulfide production (A) the urease test was negative (no color change or yellow) (B) for MIL medium, *Salmonella* spp. produces violet-colored medium (motility was positive, indole was negative, and lysine decarboxylase was positive) (C) and for SIM medium, *Salmonella* can reduce sulfur to hydrogen sulfide (hydrogen sulfide was positive, motility was positive, and indole was negative) (D)

Table 2 Results of *Salmonella* isolation from fecal swab and *Salmonella* spp. prevalence

Group of sample	No. of sample	No. of atypical Colony	No. of positive sample (%)	No. of <i>Salmonella</i> spp. isolates
Adult Swine fecal swab	80	102	32 (40%)	34
Sick swine fecal swab	20	59	13 (65%)	22
Piglet fecal swab	20	79	14 (70%)	44
Total	120	240	59 (49.17%)	100

Subsequently, we performed the antibiotic susceptibility tests by using the disk diffusion method on Muller-Hinton agar (Fig. 3). Drug susceptibility test was performed for 7 antimicrobial classes that included amphenicols (chloramphenicol), carbapenems (meropenem), cephalosporins (cefotaxime and ceftazidime), penicillin (ampicillin), quinolone (ciprofloxacin), sulfonamides (trimethoprim-sulfamethoxazole), and tetracycline. The results illustrated that *Salmonella* spp. isolates were resistant to all antibiotics used in this study, except meropenem. It was found that the highest resistance rate to ampicillin, were equal to 79.41, 81.82 and 95.45 % in isolates from adult swine, sick swine, and piglets, respectively. *Salmonella* spp. isolates were resistant to ampicillin (87%), trimethoprim-sulfamethoxazole (84%), tetracycline (62%) and chloramphenicol (61%). Based on the research of Patchanee et al., (2015b), the highest

frequency of antibiotic resistance of *Salmonella* isolates in Northern Thailand were ampicillin (83.3%) followed by tetracycline (75.7%). Simultaneously, the findings of Yue et al. (2021) in China showed that tetracycline (85.90%) and ampicillin (84.62%) had the most resistant antimicrobial agent, followed by chloramphenicol (71.80%). *Salmonella* spp. isolates from adult swine were resistant to ampicillin, trimethoprim- sulfamethoxazole, tetracycline, and chloramphenicol, which were equal to 79.41, 79.41, 64.71 and 50%, respectively. Similarly, Perron et al. (2008) reported that *Salmonella* from adult swine were resistant to common antibiotics and 65% of *Salmonella* spp. isolates showed resistance to tetracycline. Whereas *Salmonella* spp. isolates from sick swine were resistant to ampicillin, trimethoprim-sulfamethoxazole and tetracycline, which were equal to 81.82, 68.18 and 68.18%. Interestingly, *Salmonella* spp. isolates from piglets had the highest percentage of resistance. They were resistant to ampicillin, trimethoprim-sulfamethoxazole, tetracycline, chloramphenicol, and cefotaxime which were equal to 95.45, 95.45, 90.91, 56.82 and 52.27%, respectively (Table 3). Regarding the *Salmonella* spp., compared to previous studies found a lower prevalence of antimicrobial resistant of *Salmonella* spp. in swine in Thailand, no more than 20% (Pulsrikarn et al., 2012).

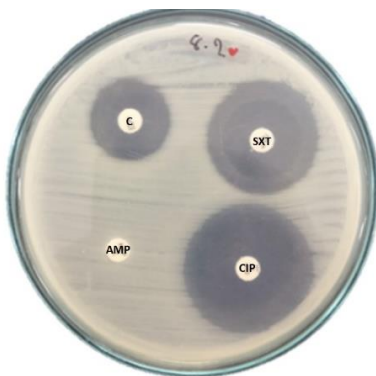


Fig. 3 Antimicrobial susceptibility test of *Salmonella* spp. performed by using the disk diffusion method

Remark: chloramphenicol (C), trimethoprim-sulfamethoxazole (SXT), ampicillin (AMP) and ciprofloxacin (CIP)

Based on results, we found that *Salmonella* spp. isolates from piglets had the highest percentage of resistance to variant of antimicrobial agents. Additionally, the probability of antimicrobial agent resistance in *Salmonella* spp. isolates from piglet has high resistance.

Table 3 Number and proportion of *Salmonella* spp. isolates resistant to different antimicrobial agents

Antimicrobial agents	Number of resistant <i>Salmonella</i> spp. isolates (%)			Total isolates (%) (n=100)
	adult swine (n=34)	sick swine (n=22)	piglet (n=44)	
Ampicillin	27 (79.41)	18 (81.82)	42 (95.45)	87 (87)
Trimethoprim-sulfamethoxazole	27 (79.41)	15 (68.18)	42 (95.45)	84 (84)
Tetracycline	22 (64.71)	15 (68.18)	25 (56.82)	62 (62)
Chloramphenicol	17 (50)	4 (18.18)	40 (90.91)	61 (61)
Ciprofloxacin	13 (38.24)	8 (36.36)	15 (34.09)	36 (36)
Cefotaxime	7 (20.59)	1 (4.55)	23 (52.27)	31 (31)
Ceftazidime	5 (14.71)	3 (18.18)	0 (0)	8 (8)
Meropenem	0 (0)	0 (0)	0 (0)	0 (0)

The study of Brun et al. (2002) speculated that young animal carry more resistant microorganisms due to increased antimicrobial exposure and physiological differences. Piglets are highly vulnerable to enteric pathogens (Lallès et al., 2007). The intestinal dysbiosis frequently seen in weaned piglets after diet change, the stress associated with changing surroundings, and the growth of swine all favor bacterial colonization by enteric pathogens. Especially, risk factors for resistance in piglets are commonly received and continuously exposed to antimicrobial agents, which raises concerns about selection for resistance (Rajic et al., 2006). Therefore, the effect of this exposure on resistance should be investigated to use in guidelines.

The *Salmonella* spp. isolates were resistant to at least one agent in three or more classes of antimicrobial agents and was defined as MDR *Salmonella*. In this study, MDR *Salmonella* was observed among 87 of 100 (87%) isolates. Seventeen different multidrug-resistant profiles were observed as shown in Table 4. Resistance to penicillin (ampicillin) and sulfonamides (trimethoprim-sulfamethoxazole) was found in MDR *Salmonella*. Furthermore, 42 (42%) isolates were resistant to antimicrobial agent in at least 4 classes. The antimicrobial resistance profiles of MDR *Salmonella* were AMP-SXT-TE-CIP, AMP-SXT-C-CTX and AMP-SXT-CTX-C-CIP which were equal to 15, 10 and 10%, respectively. Conversely, the most frequently antimicrobial resistance profiles in *Salmonella* spp. isolates from piglets were AMP-SXT-C-CTX and AMP-SXT-CTX-C-CIP. However, Phongaran et al., (2019) reported the most frequent pattern isolated from swine feces collected from slaughterhouses in nine provinces of Thailand was AMP-SXT-TET. Whereas in this study we found only 4%. In this study, we found that among the farms that recently used antimicrobials, some used antimicrobials without a prescription from

veterinarians and some farmers were unaware of the antimicrobial withdrawal time. Almost half of participants in swine farm used commercial feed. As suggested by Love et al., (2015) commercial medicated feed is likely related to the development of antimicrobial resistance. Including, the farmer was not aware of the type and dose of antimicrobial agents that was mixed in the feed.

Table 4 Antimicrobial resistance profiles of *Salmonella* spp. isolates in adult swine, sick swine, and piglet

Antimicrobial resistance profiles	Number of resistant <i>Salmonella</i> spp. isolates (%)			Total isolates (%)
	adult swine	sick swine	piglet	
TE	2 (5.88)	2 (9.09)	-	4 (4)
C	-	-	2 (9.09)	2 (2)
TE-AMP	2 (5.88)	3 (13.64)	2 (4.55)	7 (7)
TE-CAZ-CIP	3 (8.82)	-	-	3 (3)
SXT-AMP-TE	2 (5.88)	2 (9.09)	-	4 (5)
SXT-AMP-C	5 (14.71)	-	3 (6.82)	8 (8)
SXT-AMP-CIP	-	2 (9.09)	-	2 (2)
SXT-TE-C	2 (5.88)	-	-	2 (2)
AMP-SXT-CAZ-C	3 (8.82)	3 (13.64)	-	6 (6)
AMP-SXT-TE-CIP	8 (23.53)	5 (22.73)	2 (4.55)	15 (15)
AMP-SXT-TE-C	-	2 (9.09)	2 (4.55)	4 (4)
AMP-SXT-C-CTX	2 (5.88)	-	8 (18.18)	10 (10)
AMP-SXT-TE-C-CTX	3 (8.82)	-	4 (9.09)	7 (7)
AMP-SXT-TE-C-CIP	-	-	2 (4.55)	2 (2)
AMP-SXT-CTX-CIP	2 (5.88)	-	8 (18.18)	10 (10)
AMP-SXT-CTX-TE-CIP	-	1 (4.55)	-	1 (1)
AMP-SXT-CTX-TE-C-CIP	-	-	3 (6.82)	3 (3)
Total number of isolates	34	22	44	100

Remark: tetracycline (TET), cefotaxime (CTX), ceftazidime (CAZ), trimethoprim-sulfamethoxazole (SXT), ampicillin (AMP), chloramphenicol (C) and ciprofloxacin (CIP)

Antimicrobial resistance in *Salmonella* spp. from on-farm studies provides insight into the epidemiology of resistance in swine prior to transport and slaughter (Gebreyes et al., 2004). *Salmonella* resistance can also be impacted by dietary changes, stress from new surroundings, and the growth of swine. *Salmonella* spp. may provide particular concerns to food safety, as evidenced by the different rates of resistance in each stage of production, and this demonstrates that resistance is dynamic within farms. Therefore, in the future, we should look into agricultural resistance risk factors. Interventions to reduce antibiotic resistance in *Salmonella* spp. may result from identifying characteristics linked to variations in resistance between phases.

Conclusion

The resistance of *Salmonella* isolates from swine farms in Phayao Province, Thailand was described. The overall prevalence of *Salmonella* spp. in the fecal samples

in Phayao Province was 49.17%. It is higher than rates identified in swine farms from the same region, in the previous reported an occurrence of 31% (Tadee et al., 2014) and 25% (Tadee et al., 2021). The highest prevalence of *Salmonella* spp. contamination was found in the piglet fecal samples (70%), followed by sick swine fecal samples (65%) and adult swine fecal samples (40%). High resistance (>80%) was recorded toward ampicillin (87%) and trimethoprim-sulfamethoxazole (84%). MDR *Salmonella* was observed among 87 of 100 isolates (87%) and 42 isolates (42%) which were resistant to antimicrobial agent in at least 4 classes. Conversely, seventeen different multidrug-resistant profiles were observed. The most frequently found antimicrobial resistance profiles was AMP-SXT-TE-CIP. The probability of antimicrobial agent resistance in *Salmonella* spp. isolates from piglet has more resistance. Therefore, the age-specific factor study is needed to investigate reasons for differences in resistance. Likewise, further description of the associations between resistance and how resistance spreads within farms, are needed. Besides, it is time to prevent the use of antimicrobial agent in livestock to avoid the dissemination of antimicrobial resistance determinants along the food chain to avoid the transmission of foodborne pathogens to humans.

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