

Occurrence and sequence type of antimicrobial resistant *Salmonella* spp. circulating in antibiotic-free organic pig farms of northern-Thailand

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Abstract

Salmonella is one of the major foodborne pathogens, often as the result of pork consumption. Currently, with the situation of antimicrobial resistance, organic farming has been suggested as an alternative for healthier options. However, there is little evidence to support this. In this study, we investigated the occurrence of *Salmonella* circulating in local organic pig farms in northern Thailand and typed isolated clones to better understand the population structure and transmission dynamics of the underlying *Salmonella* contamination. In total, 112 samples from 11 organic pig farms were processed from October to December 2018. *Salmonella* was detected in 9 targeted farms. One-fourth (28/112) of all samples yield *Salmonella*. The positives proportion of fecal, feeder swabs, and boot swabs were found to be 32.7% (17/53), 17.7% (6/34), and 20.0% (5/25), respectively. Of the 28 positive strains, Seven *Salmonella* serotypes were identified, with *S. Rissen* being the most common (15/28; 53.6%). 89.3% (25/28), 78.6% (22/28) and 71.4% (20/28) of isolated *Salmonella* resisted against tetracycline, ampicillin, and sulfamethoxazole-trimethoprim, respectively. From multilocus sequence typing (MLST) analysis, the phylogenetic tree hinted that cross contamination within herds, point mutation of the housekeeping genes at period persisted in a herd, and sharing routes of supply chain between farms. A minimum spanning tree (MST) revealed that *Salmonella* contamination in organic pig farming is possibly linked with conventional farming. Based on the own results, strictly highly organic practices provide a safe alternative enhancing domestic consumer trust and improve public health safety.

Keywords: *Salmonella*, organic farming, antimicrobial, multilocus sequence typing, Thailand

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Introduction

Foodborne illness caused by non-typhoidal *Salmonella* is an important public health issue and is a significant economic burden worldwide (Crump and Heyderman, 2015; Padungtod and Kaneene, 2006; Pan et al., 2000; Phu et al., 2016; Scallan et al., 2011). Transmission of the pathogen to humans occurs mainly through contaminated food products from livestock (Ivic and Kocic, 2009; Jordan et al., 2006), including consumption of pig or pork products (Campos et al., 2019; Hauser et al., 2011). Clinical infection manifests with the onset of fever, followed by gastrointestinal tract disorders such as nausea, vomiting, and profuse diarrhea within 8–48 hours (Crump et al., 2015). Multidrug-resistant *Salmonella* can complicate treatment and is a serious rising issue in public health (Dechet et al., 2006), leading to a reduction in first line empirical treatment efficacy, limiting treatment choices, and prolonging illness (Pan et al., 2000).

Salmonella has been identified at all stages of the food production chain (Campos et al., 2019). Initial contamination pressure at farm level is directly related to continued contamination at subsequent levels, through slaughtering, retail, and the final cooking process (Rostagno and Callaway, 2012; Tadee et al., 2015). Pigs are often cited as the primary source of human infection, as *Salmonella* can multiply in the pig's gut and spread between animals via the fecal-oral route directly or through the environment. Healthy pigs can also carry *Salmonella* without showing any signs of infection (Futagawa-Saito, 2008); thus, increasing the opportunity for spread of *Salmonella* into the human food chain.

Currently, increasing demand has led to pig production being transformed into an intensified industry. Large numbers of antimicrobials have been implemented (Guardabassi et al., 2008) and widespread usage and misuse have contributed to the emergence of drug-resistant bacteria (Yezli and Li, 2012), one of several concerns that has fueled the increase in "organic" farming. Pigs are produced under natural management, where antibiotics and synthetic hormones are prohibited. It has set itself the goal of instituting high-quality yields with environmentally friendly production under animal welfare standards (Barański et al., 2017; Sundrum, 2001). In northern Thailand, local organic pig farms have been established for half a decade. However, data on the *Salmonella* contamination or any resistance situations have not been clearly determined.

Bacterial typing is essential for effective disease investigation and surveillance (Ferrari et al., 2017). Serotyping is based on immune reaction raised in the host and has limited discriminatory power to distinguish *Salmonella* clones (Brenner et al., 2000; Liu et al., 2001). Multilocus sequence typing is able to overcome limitations in detection and help distinguish between *Salmonella* clones and aid outbreak assessment. The method is also more reproducible and by using sequence data that can be compared between laboratories, strains can be characterized nationally or internationally (Jain et al., 2018; Yap et al., 2016). Large global studies use this method for epidemiological

studies that clearly and precisely differentiate bacterial genotypic diversity in different hosts and geographical distributions (Foxman, 2011; Liu et al., 2001).

The aims of this study were to determine the occurrence of *Salmonella* spp. circulating in organic pig farms in northern Thailand, and evaluate diversity of the pathogen. Phenotypic characteristics (serotypes and antimicrobial resistance patterns) and sequence types were measured, to understand the local population structure and dynamic propagation. The results will enhance domestic consumer trust and help improve public health safety in the region.

Materials and Methods

Sample collection: This study was conducted under ethical approval reference number R13/2561 from the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Chiang Mai University (FVM-ACUC). Pig fecal samples, feeder swabs and workers' boot swabs were collected from local organic pig farms in Chiang Mai, Chiang Rai, and Lamphun provinces, Thailand from October to December 2018. To estimate the sample size in a single proportion, the sample size of this study was determined using the Win Epi online program at (<http://www.winepi.net/uk/index.htm>) (University of Warwick, 2018). Because of entering 50% for the estimated occurrence will result in the highest sample size. Therefore, a rate of 50% was used as the "expected disease proportion (p)". Ten percent and 0.95 were selected as the "desired precision (E)" and "confidence level ($Z_{1-\alpha/2}$)" values, respectively. For the infinite population, at least 97 samples are designated. However, at the end, 112 samples were carefully chosen to provide greater accuracy and reliability of the investigation results.

Eleven open housing system pig farms with a herd size range of 30–60 pigs were randomly selected in the study. The selection criteria of all organic farms were: (1) no antibiotic or hormone usage throughout the pigs lifespan; (2) all piglets were born under the organic system; (3) use of natural feed ingredients from an organic agriculture system (either with or without commercial feed); (4) farmed according to animal welfare standards; and (5) either natural mating or artificial insemination for breeding. Approximately ten samples (5 pig feces from digital rectal examination, 3 feeder swabs, and 2 workers' boot swabs) were collected from each farm. For fecal and feeder swab samples, up to two samples/pen were chosen. All collected samples were kept in separate sterile bags and transferred to the laboratory under chilled conditions.

Salmonella isolation and identification: Isolation and identification of *Salmonella* spp. were performed following the ISO 6579:2002 Amendment 1:2007, Annex D technique, the detection of *Salmonella* spp. in animal feces, and environmental samples from the primary production stage (International Standard Organization, 2002). 25 g of fresh fecal samples and swab samples were added to 225 ml of and 9 ml of buffered peptone water (BPW) (Merck, Germany) as pre-enrichment media. The mixtures were homogenized for 2 min and incubated at 37 °C for 24

h. An aliquot of 0.1 ml was then dropped on Modified Semi-solid Rappaport-Vassiliadis (MSRV) (Oxiod, United Kingdom) plates and incubated at 42 °C for 24 h. The culture material surrounded by a turbid ring was then streaked on Xylose Lysine Deoxycholate (XLD) agar (Oxiod, United Kingdom), and Brilliant Green Phenol Red Lactose Saccharose (BPLS) agar (Merck, Germany) with incubation at 37 °C for 24 h. Presumptive *Salmonella* colonies were additionally checked by biochemical measurement, including testing of triple sugar iron (TSI) (Oxiod, United Kingdom), urease, and motile indole lysine (MIL) decarboxylase (Merck, Germany). *Salmonella* positive samples were recorded. Only one isolate from each sample was chosen for further investigation.

Serotyping and Antimicrobial susceptibility testing:

All detected *Salmonella* spp. were serotyped by serum-agglutination according to the White-Kauffmann-Le Minor scheme (Brenner *et al.*, 2000). Antimicrobial susceptibility testing was determined by agar disk diffusion techniques with ten panels of antimicrobial agents, comprising amoxicillin-clavulanic acid 20/10 µg, ampicillin (AMP) 10 µg, chloramphenicol (C) 30 µg, ciprofloxacin (CIP) 5 µg, cefotaxime (CTX) 30 µg, nalidixic acid (NA) 30 µg, norfloxacin (NOR) 10 µg, streptomycin (S) 10 µg, sulfamethoxazole-trimethoprim (SXT) 23.75/1.25 µg, and tetracycline (TE) 30 µg (CLSI, 2018). Strains with intermediate resistance profiles were recorded as resistant strains to avoid underestimation.

Statistical analysis: *Salmonella* occurrence with their 95% confidence levels were determined by descriptive statistics. Comparisons between groups of sample types were performed using Fisher's exact test. All analyses were accomplished using Epi Info™, version 7 (Centers for Disease Control and Prevention, USA). Statistical significance levels were measured at $p < 0.05$.

Multilocus sequence typing: All *Salmonella* strains detected were genotyped into sequence types (STs) by the MLST technique. Total genomic DNA was extracted according to the protocol described by Liu *et al.* (2011). Seven housekeeping genes, including *aroC* (chorismate synthase), *dnaN* (DNA polymerase III beta subunit), *hemD* (uroporphyrinogenIII cosynthase), *purE* (phosphoribosylaminoimidazole carboxylase), *sucA* (alpha ketoglutarate dehydrogenase), *hisD* (histidinol dehydrogenase), and *thrA* (aspartokinase I/homoserine dehydrogenase) were taken for MLST profiling. Polymerase Chain Reaction (PCR) amplification of all seven genes was accomplished using the methods previously described by Achtman *et al.* (2012). Then, the PCR products were sent for sequencing to the Macrogen Service Center, Republic of Korea. All sequences obtained in each gene were transformed into allele number and compiled for sequence type (ST) data from the database of <http://mlst.warwick.ac.uk/mlst/> (University of Warwick Enterobase, 2020).

Using Bionumerics® software, version 7.6 (Applied Maths, Belgium), by the unweighted pair group method with arithmetic mean algorithms (UPGMA) through individual similarity matrices, cluster

analyses of the seven genes were executed. Local epidemiological results of strains currently circulating in organic pig farms in the study area were displayed in phylogenetic networks. In addition, to develop understanding of evolution and population structure, minimum spanning tree (MST) analysis was used to analyze the genetic relationship between *Salmonella* stains obtained from this study together with the geographically and temporally matched strains previously submitted to the MLST database (University of Warwick Enterobase, 2020), Warwick Medical School, University of Warwick. Those included were Thai strains recovered from pig ($n = 218$), human ($n = 42$), plant ($n = 37$), frozen food ($n = 30$), aquatic animal ($n = 16$), dried food ($n = 13$), poultry ($n = 9$), soil ($n = 6$), invertebrate ($n = 2$), cooked food ($n = 1$), and marine mammal ($n = 1$), submitted during 2009–2018. The STs, which are closely associated in loci characteristics, were demonstrated close together.

Results

***Salmonella* occurrence:** From October to December 2018, 112 fecal and environmental samples were collected from 11 local organic pig farms (farms A–K). Eight to 13 samples were obtained from each. One-fourth (28/112; 95% CI: 17.30–34.07%) of all samples yield *Salmonella*. The positives proportion of fecal, feeder swabs, and boot swabs were found to be 32.08% (17/53; 95% CI: 19.92–46.32%), 17.65% (6/34; 95% CI: 6.76–34.53%), and 20.00% (5/25; 95% CI: 6.83–40.70%), respectively. There was no association between *Salmonella* frequencies detected and types of sample taken. *Salmonella* contamination was detected in nine of the eleven farms tested, with only two farms (farms F and K) negative. Between 10–20% of the samples obtained from six farms (farms B, D, E, G, H, and I) were positive to *Salmonella*. For the three remaining farms (farms A, C, and J), 50–60% detection rates were distributed.

***Salmonella* sero-distribution:** Seven *Salmonella* serotypes were identified in the study. *S. Rissen* was the most common (15/28; 53.57%), followed by monophasic *S. Typhimurium* 1,4,[5],12: i: - (7/28; 25%), *S. Weltevreden* (2/28; 7.14%), and one strain (1/28; 3.57%) each of *S. Hvittingfos*, *S. Krefeld*, *S. Stanley*, and *S. Typhimurium*.

Antimicrobial susceptibility testing: Of all 28 strains taken from *Salmonella* positive samples, only one (3.57%) was susceptible to all antimicrobials tested, while 24 strains (85.71%) were resistant to at least three different antimicrobials, classifying them as multidrug-resistant (MDR) strains. Resistance of *Salmonella* from the current study could be found concerning eight of ten antimicrobials tested. No resistance was observed to ciprofloxacin and norfloxacin. Most strains were resistant to tetracycline (25 strains; 89.29%), followed by ampicillin (22 strains; 78.57%) and sulfamethoxazole-trimethoprim (20 strains; 71.43%) (Figure 1).

Multilocus sequence typing: Sequencing data of seven housekeeping genes of all strains were submitted to the *Salmonella* spp. definition database to query allelic numbers and corresponding STs. In total, 12 different MLST genotypes were assigned. Six of them were matched with the previously described in MLST database (University of Warwick Enterobase, 2020), could be assigned in ST number. The majority were of ST469 ($n = 14$), followed by three strains of ST34 and two strains of ST365. Six others could not be assigned a ST, highlighting the diversity of *Salmonella* isolates found on these farms. Variation in the *sucaA* and/or *hisD* or *thrA* loci enabled assignment to ST complex or clonal complex (CC) level. Five strains were assigned to CC1, and the other one was CC66. The genetic relationship between all 28 *Salmonella* strains was explored using sequence variation from the seven housekeeping genes with UPGMA algorithms (Figure 2).

Six clusters were determined with two or more MLST gene differences, representing different CCs. The strains grouped in the dominant serovar Rissen ST469 were mostly obtained from three nearby farms (farms A-C, which each has a similar zip-code), and two identical strains were also obtained from farms approximately 30-70 km away from each other (farms E and I). Besides, strains grouped in Weltevreden ST365 were also obtained from farms spread more than 30 km apart (farms A and I).

Serovar Rissen ST469 from farm A was recovered from feeder swabs and fecal samples. Similar to the

ST34 strains from farm J, the strains were recovered from a boot swab and fecal samples. Moreover, in farms A, B, E, H, I, and J, at least two ST characteristics were identified.

In order to better understand the genetic relatedness of the pathogen, a minimum spanning tree (MST) was constructed to analyze the 28 strains alongside 375 Thai strains previously submitted to the MLST database in the ten-year period, 2009-2018 (Figure 3). A total of 403 Thai strains was constructed describing a population from 11 different hosts. Seventy-one STs were distributed. More than two-fifths of the strains were closely related and shared allelic profiles in at least five loci, with similar CCs shaded in gray. ST34 was the most frequently detected, followed by ST465, ST365, and ST29. Members grouped in the top four STs were recovered from more than one host origin, such as organic pig, pig, plant, soil, poultry, food, and human. For the members grouped in ST365, one was obtained from an invertebrate. On the other hand, some STs were unique to a single host origin; ST185, ST410, ST590 were specific to plant sources. Similarly, ST1 and ST292 were specific to human and aquatic animals, respectively.

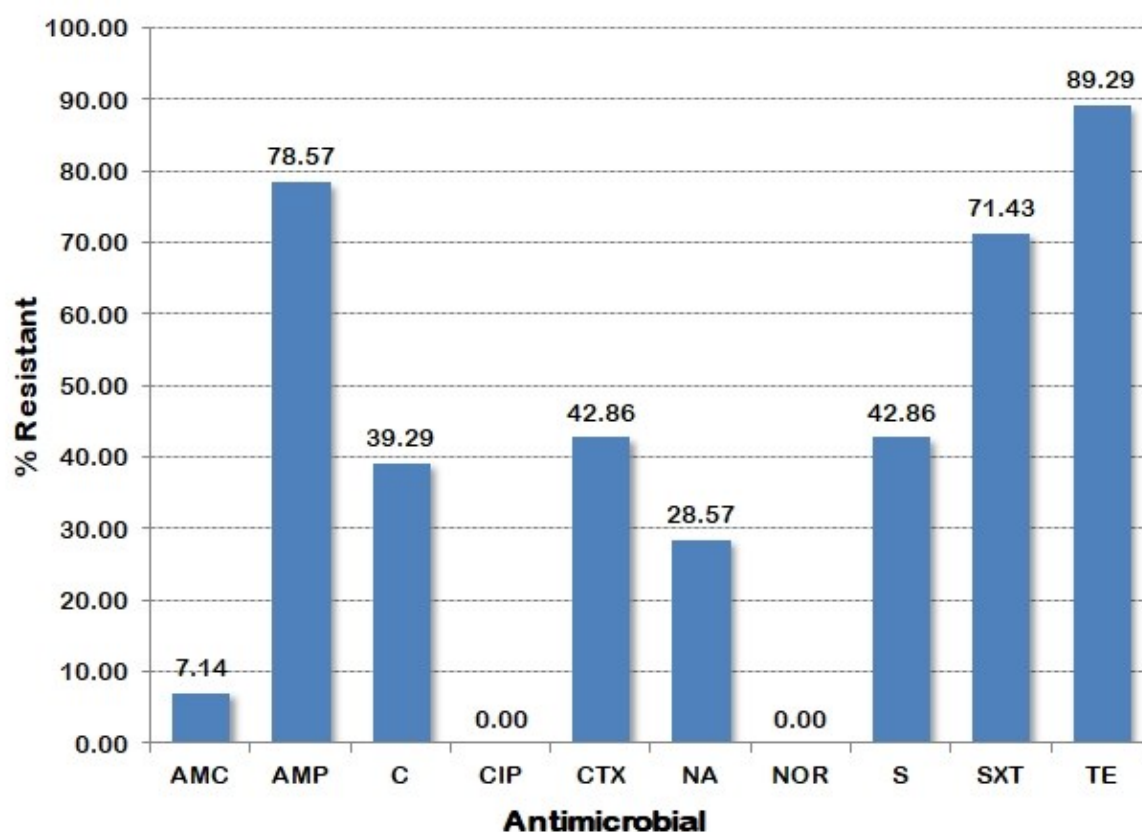


Figure 1 Antimicrobial resistance rates of *Salmonella* strains isolated from local organic pig farming in northern Thailand. Antibiotic abbreviations: amoxicillin-clavulanic acid (AMC); ampicillin (AMP); chloramphenicol (C); ciprofloxacin (CIP); cefotaxime (CTX); nalidixic acid (NA); norfloxacin (NOR); sulfamethoxazole-trimethoprim (SXT); streptomycin (S); tetracycline (TE)

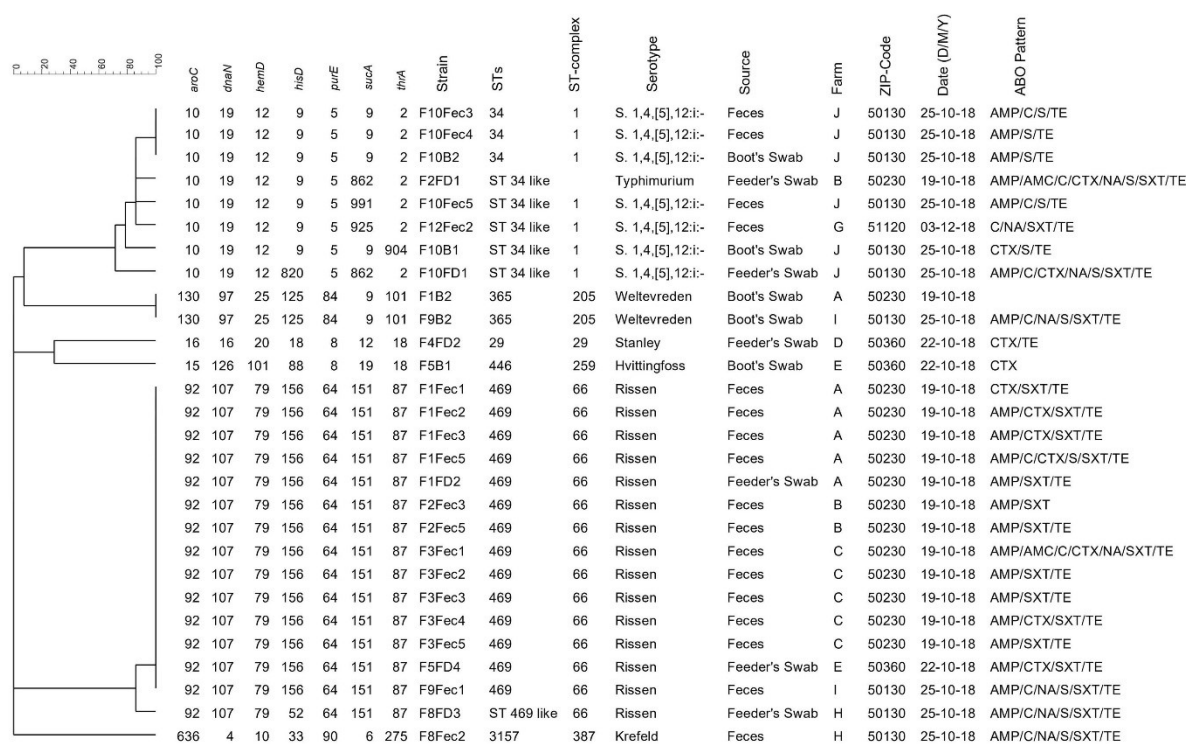


Figure 2 Dendrograms generated using UPGMA algorithms based on MLST profiles with the phenotypic characterization and epidemiological data of *Salmonella* circulating on organic pig farms in northern Thailand.

Discussion

This is the first cross-sectional study of *Salmonella* spp. circulating in organic pig farming in the northern region of Thailand. We identify overall positives of 25% (95% CI: 18–35%), which is only slightly lower than rates identified in conventional pig farms from the same region during the same time period (Tadee *et al.*, 2014), that reported an occurrence of 31% (95% CI: 27–33%). This is far higher than rates identified in other similar study on organic farms from other country in Asia, where an occurrence of 8% (95% CI: 6–10%) was reported in a study of Korean organic pig farms by Tamang *et al.* (2015). These high levels of *Salmonella* contamination suggest that there is a breakdown in organic practices in rural northern Thailand.

All samples acquired from farms F and K were negative. However, only ten samples were collected from each farm, so these farms cannot yet be considered *Salmonella*-free, exactly. Although all the farms followed organic principles, at the present time farm K is the only one in northern Thailand that has received official accreditation from the Thai government, suggesting that standards may be higher on these farms. Organic products may provide an alternative for health-conscious local consumers. Of that farm, one hundred percent use of natural feed ingredients is likely beneficial (Rostagno and Callaway, 2012), with a direct effect on the pigs' gut microbiota (García-Feliz *et al.*, 2009; Rajić *et al.*, 2007). The use of low pH fermented grain and natural organic feed stuff are act as a prebiotic, promoting growth of lactic acid bacteria to the detriment of the preferred conditions for *Salmonella* (Farzan *et al.*, 2006; Hedemann *et al.*, 2005).

As one might expect following comparison among sample types, *Salmonella* positive percentage from fecal samples tended to be higher than the value acquired from boot and feeder swab samples. Expectedly, *Salmonella* usually multiplies in pig's gut (Futagaea-Saito *et al.*, 2008). Ingested *Salmonella* can be shed via feces within 2 h, without any clinical symptom and spread to the wider environment, such as stable floors or feeders (Hauser *et al.*, 2011; Rostagno and Callaway, 2012). Consequently, hygienic practices in stable cleaning, boot disinfecting, and feed management are very important in reducing the risk of contamination (Rajić *et al.*, 2007). However, other on-farm stressor factors including handling for mating, transportation as well as high density in the rearing area are also considered as multifactorial effects that increase the chance of *Salmonella* shedding (García-Feliz *et al.*, 2009). For that reason, effective herd management allowing natural behavior expression should be fulfilled. This could also be considered an explanation for why *Salmonella* could not be found on farms F and K, which seemingly had higher hygiene standards than the other farms, even though natural feeds were not 100% implemented on farm F.

All 28 detected *Salmonella* strains were susceptible to norfloxacin and ciprofloxacin, both of which are fluoroquinolones, which are necessary in parenteral treatment of severe gastroenteritis in human cases (Bruzzese *et al.*, 2018; Malik, 2017). Fluoroquinolones remain the most effective antimicrobials for pig related salmonellosis in humans. However, nine-tenths of strains tested in the study were MDR. High resistance rates of antimicrobials recently used in livestock production, such as ampicillin, tetracycline, or sulfamethoxazole-trimethoprim were found. This is

consistent with reports on *Salmonella* isolated from conventional pig farms in northern Thailand that also revealed high levels of resistance to these drugs (Chuanchuen and Padungtod, 2009; Tadee et al., 2014). Generally, antimicrobial usage is currently prohibited in all organic farms selected for this study. Spread of MDR *Salmonella* seems not to be the effect of selective pressure in the antibiotic-free organic pig farming. This finding illustrates the complicated and widespread nature of resistance in the farm community (Rostagno

and Callaway, 2012). Breeders reared on conventional farms that enter the organic system may already carry drug-resistant *Salmonella* in their guts, providing an opportunity for transmission and infection. Accordingly, it is important to note that a resistance has also emerged in antimicrobial-free herds (Österberg et al., 2016). In relation to farms F and K, breeders are produced on farm, thus limiting the possibility of pathogen transmission from off-farm sources.

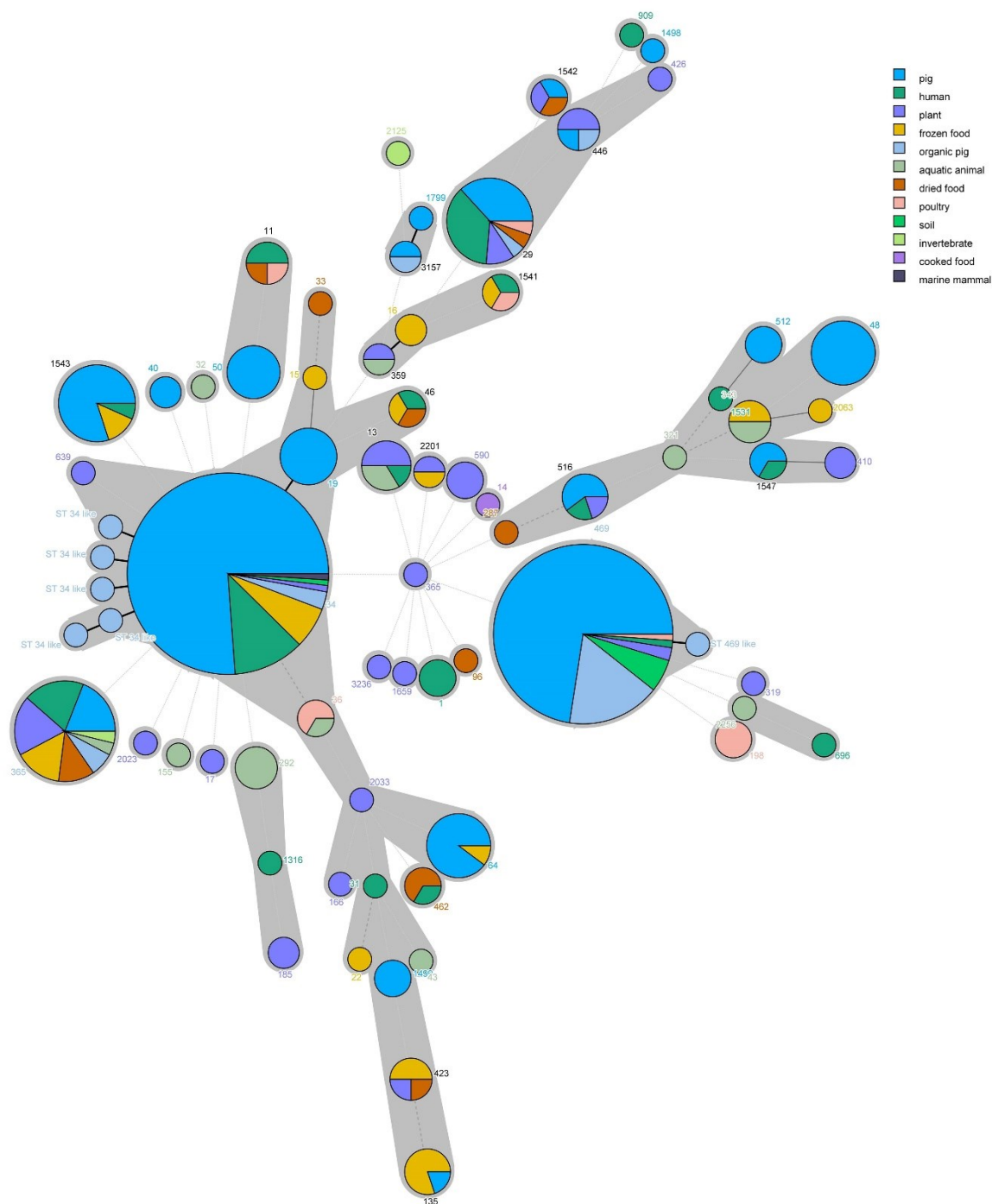


Figure 3 Minimum spanning tree (MST) analysis of 403 Thai population *Salmonella* strains recovered from various host origins during 2009–2018.

Size of circles indicates the number of strains grouping in the same STs. Branch styles coding: thick black solid lines connect ST types with one locus difference; thin black solid lines connect ST types with double and three loci differences; gray dashed lines connect four loci differences; gray dotted lines connect more than four loci differences, respectively. Gray shaded areas indicate at least five shared loci between STs (grouping in common ST complexes)

Shared STs belonging to different sample types were identified, suggesting a common source of contamination. Within herds, cross transmission can occur via carriers or any contaminated materials and the probability of re-infection could occur continuously (Rajić *et al.*, 2007; Rostagno and Callaway, 2012). If we consider the geography of our collection, 12 strains of ST469 were recovered from three different farms (farms A, B, and C) located in nearby areas, with the same zip-code. Farm staff, environmental contamination, and the sharing of routes for pig transportation could play a significant role in dissemination. Nevertheless, associations of spatial relatedness were also noted at farms located far apart (farms E and I). As a previously noted, common supply chains such as breeders, gilts, or feed stuffs might be inferred as an important route. *S. Rissen* was the most common serotype detected and is a dominant clone *Salmonella* contamination of pig farms in northern Thailand over the last 15-years (Padungtod and Kaneene, 2006; Tadee *et al.*, 2014). In view of this, *Salmonella* contamination of both conventional and organic pig farms is probably linked.

Diversity in MLST genotypes in some farms provides evidence that multiple strains are present within a single herd. Farms A, B, E, H, and I harbored at least two genotypical characteristics of *Salmonella*. This may represent different sources of infection. For another explanation, variation in one or two alleles can be explained by point mutations in the housekeeping genes during persistent colonization of the herd. This is potentially what we see with the ST34 strains from farm J, where only one to three nucleotide changes can be identified in the *sucA* gene. Using the allele sequence comparison analysis function of the pubMLST *Salmonella* spp. MLST database (Jolley *et al.*, 2018), point mutations can be specified: 155A>G of *thrA* alters *thrA_2* to *thrA_904*, with 99.8% identity. With the same identity, a variation 127G>A of *sucA* shifts *sucA_9* to *sucA_862*. Additionally, variations 15C>T, 18A>G, and 54C>T of *sucA* make over *sucA_9* to *sucA_925* with only 99.4% identity. This allelic profile has not yet been referenced in the database and a new ST profile will need to be added.

As part of expanding knowledge of regional epidemiology, results of the genetic relatedness of Thai *Salmonella* strains obtained from several host origins from ten collection years were displayed in MST. A diverse set of 403 *Salmonella* spp. strains were evaluated. Sixty percent of them were pig associated strains, supporting a strong interest in *Salmonella* contamination of pork products in the region. Strains grouped in the majority STs were obtained from the host origins linked with human food chain. From the finding, pig, chicken and aquatic animals could be important source of salmonellosis. They are denoted as the primary source of infection and can spread the pathogen to environment and subsequent level (Compos *et al.*, 2019; Rostagno and Callaway, 2012). In addition, most strains derived from organic pig farms were clonally related to the pig associated strains from the conventional farming system. Predominant types, especially ST34 and ST469 have been spread into pig farms across the country and have been a source of contamination in the pig population for over a decade.

One of the ST365 strains was recovered from invertebrate, which could be an additional potential reservoir of salmonellosis. Moreover, ST1 was found to be unique in the human host origin. Not surprisingly, all strains belonging to ST1 were derived from *Salmonella* Typhi, which is renowned as the host specific serotype for humans (Yap *et al.*, 2016). For STs which were unique to other origins, the low numbers of strains submitted does not reflect that these cannot be transmitted or spread to the others. This requires further study to develop a complete understanding of their epidemiology.

Consumers demand choice and healthier options. Organic products aim to provide increased food security, animal wellbeing as well as consideration of the effects on environmental sustainability. However, in this study *Salmonella* was detected in nine of the eleven farms tested. All farms tested claimed to follow organic principles, but only one had received official Thai government certification - from which no *Salmonella* contamination was detected. Although *Salmonella* occurrence was slightly lower in these organic farms, contamination with multidrug resistant clones suggests that domestic consumers should still be wary and that subsequent meat products still pose a public health risk.

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Conflicts of Interest: The authors declare no conflict of interest.

References

- Achtman M, Wain J, Weill FX, Nair S, Zhou Z, Sangal V, Krauland MG, Hale JL, Harbottle H, Uesbeck A, Dougan G, Harrison LH and Brisse S 2012. *S. enterica* MLST Study Group. Multilocus sequence typing as a replacement for serotyping in *Salmonella enterica*. PLoS Pathog. 8: e1002776.
- Barański M, Rempelos L, Iversen PO and Leifert C 2017. Effects of organic food consumption on human health; the jury is still out. Food. Nutr. Res. 61: 1287333.
- Brenner FW, Villar RG, Angulo FJ, Tauxe R and Swaminathan B 2000. *Salmonella* nomenclature. J Clin Microbiol. 38: 2465-2467.
- Bruzzese E, Giannattasio A and Guarino A 2018. Antibiotic treatment of acute gastroenteritis in children. F1000Res. 17: 193.
- Campos J, Mourão J, Peixe L and Antunes P 2019. Non-typhoidal *Salmonella* in the pig production chain: a

- comprehensive analysis of its impact on human health. *Pathogens*. 8: E19.
- Chuanchuen R and Padungtod P 2009. Antimicrobial resistance genes in *Salmonella enterica* isolates from poultry and swine in Thailand. *J. Vet. Med.* 71: 1349-1355.
- Clinical and Laboratory Standards Institute (CLSI) 2018. Performance standards for antimicrobial disc susceptibility test 13th ed. Pennsylvania: Wayne. 72 pp.
- Crump JA and Heyderman SR 2015. A perspective on invasive *Salmonella* disease in Africa. *Clin. Infect.* 61: S235-S240.
- Crump JA, Sjölund-Karlsson M, Gordon MA and Parry CM 2015. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. *Clin. Microbiol. Rev.* 28: 901-937.
- Dechet AM, Scallan E, Gensheimer K, Hoekstra R, Gunderman-King J, Lockett J, Wrigley D, Chege W, Sobel J 2006. Outbreak of multidrug-resistant *Salmonella enterica* serotype Typhimurium Definitive Type 104 infection linked to commercial ground beef, northeastern United States, 2003-2004. *Clin. Infect. Dis.* 42: 747-752.
- Farzan A, Friendship RM, Dewey CE, Warriner K, Poppe C and Klotins K 2006. Prevalence of *Salmonella* spp. on Canadian pig farms using liquid or dry-feeding. *Prev. Vet. Med.* 73: 241-254.
- Ferrari RG, Panzenhagen PHN and Conte-Junior CA 2017. Phenotypic and genotypic eligible methods for *Salmonella* Typhimurium source tracking. *Front. Microbiol.* 8: 2587.
- Foxman B 2011. *Molecular Tools and Infectious Disease Epidemiology*, 1st ed. Massachusetts: Academic Press. 215 pp.
- Futagawa-Saito K, Hiratsuka S, Kamibeppu M, Hirotsawa T, Oyabu K and Fukuyasu T 2008. *Salmonella* in healthy pigs: prevalence, serotype diversity and antimicrobial resistance observed during 1998-1999 and 2004-2005 in Japan. *Epidemiol. Infect.* 136: 1118-1123.
- García-Feliz C, Carvajal A, Collazos JA and Rubio P 2009. Herd-level risk factors for faecal shedding of *Salmonella enterica* in Spanish fattening pigs. *Prev. Vet. Med.* 91: 130-136.
- Guardabassi L, Jensen LB and Kruse H 2008. *Guide to antimicrobials used in animals*, 1st ed. Oxford: Blackwell Publishing. 223 pp.
- Hauser E, Hebner F, Tietze E, Helmuth R, Junker E, Prager R, Schroeter A, Rabsch W, Fruth A and Malorny B 2011. Diversity of *Salmonella enterica* serovar Derby isolated from pig, pork and humans in Germany. *Int. J. Food Microbiol.* 151: 141-149.
- Hedemann MS, Mikkelsen LL, Naughton PJ and Jensen BB 2005. Effect of feed particle size and feed processing on morphological characteristics in the small and large intestine of pigs and on adhesion of *Salmonella enterica* serovar Typhimurium DT12 in the ileum in vitro. *J. Anim. Sci.* 83: 1554-1562.
- International Standard Organization 2002. *Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Salmonella spp.* ISO 6579:2002(E), 4th ed. Geneva: International Standard Organization. 30 pp.
- Tadee P. et al. / *Thai J Vet Med.* 2021. 51(2): 311-319.
- Ivic Kolevska S and Kocic B 2009. Food contamination with *Salmonella* species in the Republic of Macedonia. *Foodborne. Pathog. Dis.* 6: 627-630.
- Jain P, Sudhantirakodi S, Chowdhury G, Joshi S, Anandan S, Ray U, Mukhopadhyay A and Dutta S 2018. Antimicrobial resistance, plasmid, virulence, multilocus sequence typing and pulsed-field gel electrophoresis profiles of *Salmonella enterica* serovar Typhimurium clinical and environmental isolates from India. *PLoS One.* 13: e0207954.
- Jolley KA, Bray JE and Maiden MCJ 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res.* 3: 124.
- Jordan E, Egan J, Dullea C, Ward J, McGillicuddy K, Murray G, Murphy A, Bradshaw B, Leonard N, Rafter P and McDowell S 2006. *Salmonella* surveillance in raw and cooked meat and meat products in the Republic of Ireland from 2002 to 2004. *Int. J. Food Microbiol.* 112: 66-70.
- Liu WB, Liu B, Zhu XN, Yu SJ and Shi XM 2001. Diversity of *Salmonella* isolates using serotyping and multilocus sequence typing. *Food Microbiol.* 28: 1182-1189.
- Malik OAA 2017. Role of antimicrobials in the treatment of adult patients presenting to the emergency department with acute gastroenteritis - A mini review. *Pak. J. Med. Sci.* 33: 488-492.
- Österberg J, Wingstrand A, Nygaard JA, Kerouanton A, Cibir V, Barco L, Denis M, Aabo S and Bengtsson B 2016. Antibiotic resistance in *Escherichia coli* from pigs in organic and conventional farming in four European countries. *PLoS One.* 11: e0157049.
- Padungtod P and Kaneene JB 2006. *Salmonella* in food animals and humans in northern Thailand. *Int. J. Food Microbiol.* 108: 346-354.
- Pan H, Paudyal N, Li X, Fang W and Yue M 2018. Multiple food-animal-borne route in transmission of antibiotic-resistant *Salmonella* Newport to humans. *Front. Microbiol.* 9: 23.
- Phu Huong Lan N, Le Thi Phuong T, Nguyen Huu H, Thuy L, Mather AE, Park SE, Marks F, Thwaites GE, Van Vinh Chau N, Thompson CN and Baker S 2016. Invasive non-typhoidal *Salmonella* Infections in Asia: clinical observations, disease outcome and dominant serovars from an infectious disease hospital in Vietnam. *PLoS Negl. Trop. Dis.* 10: e0004857.
- Rajić A, O'Connor BP, Deckert AE, Keenlside J, McFall ME, Reid-Smith RJ, Dewey CE and McEwen SA 2007. Farm-level risk factors for the presence of *Salmonella* in 89 Alberta swine-finishing barns. *Can. J. Vet. Res.* 71: 264-270.
- Rostagno MH and Callaway TR 2012. Pre-harvest risk factors for *Salmonella enterica* in pork production. *Food Res. Int.* 45: 634-640.
- Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL and Griffin PM 2011. Foodborne illness acquired in the United States--major pathogens. *Emerg. Infect. Dis.* 17: 7-15.
- Sundrum A 2001. Organic livestock farming: A critical review. *Livest. Prod. Sci.* 63: 207-214.

- Tadee P, Boonkhot P, Pornruangwong S and Patchanee P 2015. Comparative phenotypic and genotypic characterization of *Salmonella* spp. in pig farms and slaughterhouses in two provinces in northern Thailand. PLoS One. 10: e011658.
- Tadee P, Kumpapong K, Sinthuya D, Yamsakul P, Chokesajjawatee N, Nuanualsuwan S, Pornsukarom S, Molla BZ, Gebreyes WA and Patchanee P 2014. Distribution, quantitative load and characterization of *Salmonella* associated with swine farms in upper-northern Thailand. J. Vet. Sci. 15: 327-334.
- Tamang MD, Gurung M, Nam HM, Moon DC, Kim SR, Jang GC, Jung DY, Jung SC, Park YH and Lim SK 2015. Prevalence and characterization of *Salmonella* in pigs from conventional and organic farms and first report of *S. serovar* 1,4,[5],12:i:- from Korea. Vet. Microbiol. 178: 119-124.
- University of Warwick 2018. "Enterobase: *Salmonella enterica* MLST Database." [Online]. Available: <http://mlst.warwick.ac.uk/mlst/>. Accessed January 22, 2020.
- University of Zaragoza (n.d.). "Win Epi: Sample size: Estimate Percentage." [Online]. Available: <http://www.winepi.net/uk/index.htm>. Accessed March 21, 2018.
- Yap KP, Ho WS, Gan HM, Chai LC and Thong KL 2016. Global MLST of *Salmonella* Typhi revisited in post-genomic era: genetic conservation, population structure, and comparative genomics of rare sequence types. Front. Microbiol. 7: 270.
- Yezli S and Li H 2012. Antibiotic resistance amongst healthcare-associated pathogens in China. Int. J. Antimicrob. Agents. 40: 389-397.