Original Article

Molecular epidemiology and antimicrobial resistance of *Salmonella* spp. isolated from broilers and pigs at slaughterhouses in Thailand and China

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Abstract

This study aimed to compare the antimicrobial resistance patterns and DNA restriction patterns by Pulsed-Field Gel Electrophoresis (PFGE) between *Salmonella* spp. isolated from broilers and pigs in Thailand and China. One hundred and sixty six isolates were collected from broilers and pigs at slaughterhouses in Khon Kaen, Thailand (n=106) between February to August 2017 and in Chongqing, China (n=60) between March to October 2015. Antimicrobial susceptibility of the isolates was determined using the disk diffusion method with nine antimicrobial agents. Genotypic diversity of the isolates used PFGE of *Xba*I-digested chromosomal DNA to determine. Of 166 *Salmonella* isolates, 37 serotypes were identified. The Thai isolates were composed of 18 serotypes, of which *S.* Rissen was most common (34%). The Chinese isolates consisted of 23 serotypes, of which *S.* Derby was most common (22%). The antimicrobial resistance profiles of *Salmonella* isolates demonstrated that 38% (63/166) of the isolates were multidrug resistance (MDR), with 20.8% (22/106) of the Thai isolates and 68.3% (41/60) of the Chinese isolates being identified as MDR. *Salmonella* Typhimurium and *S.* Give isolated from Thailand and China were found to be clonally unrelated. It is noteworthy that a close genetic relationship, at more than 90% similarity, was observed between *S.* Rissen isolated from pigs in China and Thailand. Based on these results, better surveillance systems for *Salmonella* spp. should be implemented, and the use of antibiotics in food animal production should be more tightly regulated in both Thailand and China. The monitoring program of *Salmonella* dissemination should stay vigilant for *S.* Rissen between Thailand and China.

Keywords: *Salmonella* spp., antimicrobial resistance, PFGE, Thailand, China

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Introduction

Salmonella spp. is an important foodborne pathogen causing 93.8 million cases of gastroenteritis globally each year (Majowicz et al., 2010). Pork and poultry meats are among the major sources of foodborne salmonellosis. *Salmonella* spp. can colonize the gastrointestinal tract of animals and cause enteritis. However, infected animals are commonly found to carry and shed the pathogen for a period of time without presenting any clinical signs. *Salmonella* spp. can, thus, enter the food chain during the slaughtering process. Contamination of carcasses can occur during the poor evisceration step and is linked to cross-contamination to humans through consumption of contaminated meat.

The emergence of antimicrobial resistant bacteria (AMR) caused by improper use of antibiotics poses global health threats to both humans and animals (Landers et al., 2012). Antimicrobial resistance was observed in *Salmonella* spp. in the early 1990s, and it has dramatically increased over the past decade (Guerra et al., 2014; Clemente et al., 2015). Antibiotic drugs are used in livestock production for many purposes, including treatment, prevention and control of bacterial infections, and growth promoters (Castanon, 2007). A relationship between the practice of in-feed antimicrobials for animals and the rising problem of drug-resistant bacterial infections in humans has been demonstrated in prior studies (Witte, 2000). Overuse and misuse of antibiotics are the important keys which drive the evolution of antibiotic resistance in bacteria (Read et al., 2014). Antimicrobial resistance genes can be transferred between bacteria by horizontal gene transfer and mobile genetic elements, leading to the emergence and spread of antibiotic resistant bacteria in new geographic locations or new populations (Ventola, 2015).

Travel-associated *Salmonella* infections have become a problem globally. There are several studies indicating that human salmonellosis is related to people who have traveled overseas in the week before they became ill (Hendriksen et al., 2008; Sirichote et al., 2010). Thailand is a famous travelling destination for travelers all over the word. In 2016, there were more than 8,770,000 Chinese tourists travelling to Thailand (Manarungsan, 2009). In addition, Thailand-China bilateral trade has recently been developed and agricultural products, including live animals and meat (Thailand Ministry of Commerce, 2017), are the main products in the bilateral trade. Thus, there is an increase in the risk of foodborne pathogens spreading between Thailand and China (Pornruangwong et al., 2008, Manarungsan, 2009). In view of the current challenges over the emergence of antimicrobial resistant bacteria and a greater risk of pathogens spreading through travel and trade, the objective of this study was to determine antimicrobial resistance patterns and genetic relatedness of *Salmonella* isolates from broilers and pigs at slaughterhouses in Khon Kaen, Thailand and Chongqing, China. Comparative characterization of *Salmonella* isolates from Thailand and China would be useful for planning effective interventions for the control of *Salmonella* and AMR spread.

Materials and Methods

Sample collection: From February to August 2017, 400 samples were randomly collected from broilers (n=200) and pigs (n=200) at 3 local slaughterhouses (2 for broilers, 1 for pigs) located in Khon Kaen Province, Thailand. Cloacal and rectal swabs were collected after stunning and the swabs were put in Cary-Blair transport medium (Oxoid, England) tubes individually. All samples were kept on ice and transported to laboratory.

The Animal Science and Technology College, Southwest University, China provided 60 *Salmonella* isolates. The isolates were collected from broilers (n=21) and pigs (n=39) at 7 local slaughterhouses in Chongqing, China from March to October 2015.

The study was reviewed and approved by the Institutional Animal Care and Use Committee of Khon Kaen University, No. IACUC-KKU-97/60.

Salmonella isolation and identification: Isolation and identification of the *Salmonella* isolates were performed using standard method ISO 6579-1 (ISO, 2007). Briefly, the swabs were put in 9 ml Buffer Peptone Water (BPW, Difco, France) and incubated at 37°C for 24 hours. Bacterial suspensions were then placed on Modified Semisolid Rappaport Vassiliadis medium (MSRV, Difco, France) and incubated at 37°C for 24 hours. Presumptive colonies were streaked on Xylose Lysine Deoxycholate Agar (XLD, Difco, France) and incubated at 37°C for 24 hours. Colonies of presumptive *Salmonella* were selected and confirmed by biochemical tests, including Triple Sugar Iron Agar (TSI, Difco, France) and Motility Indole Lysine Medium (MIL, Difco, France). Serotyping was performed by slide agglutination based on the Kaufman-White scheme using a commercially available antiserum (ECDC 2012; S&A Reagents Lab, Bangkok, Thailand).

Antimicrobial susceptibility testing: The Kirby-Bauer disk diffusion method was carried out according to the Clinical and Laboratory Standards Institute (CLSI) M100 27th standard (CLSI, 2017). The nine antimicrobial agents included ampicillin (AMP, 10 µg), amoxycillin/clavulanate (AMC, 20/10 µg), chloramphenicol (CHL, 30 µg), ciprofloxacin (CIP, 5 µg), cefotazidime (CAZ, 30 µg), nalidixic acid (NAL, 30 µg), norfloxacin (NOR, 10 µg), trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 µg), and tetracycline (TET, 30 µg). *Escherichia* coli ATCC-25922 were used as quality control strains.

Pulsed-Field Gel Electrophoresis (PFGE) genotyping: Pulsed-Field Gel Electrophoresis (PFGE) was performed following the PulseNet protocol from the Centers for Disease Control and Prevention (CDC, 2017). Briefly, the *Salmonella* isolates were grown on Mueller-Hinton agar (MHA) at 37°C for 14-18h. Cell Suspension Buffer (100 mM Tris: 100 mM EDTA, pH 8.0) was used to suspend and adjust the bacterial concentration to an optical density (OD600) of 1.0. Cell Lysis Buffer (50 mM Tris: 50 mM EDTA, pH 8.0 + 1% Sarcosyl) and Proteinase K (SIGMA Life Science, SL, USA) were used to lyse the bacterial cells embedded in

agarose plugs, and genomic DNA was then digested with *Xba*I restriction enzyme (New England Biolabs Ipswich, MA, USA). *Salmonella enterica* serovar Breanderup H9812 was used as a molecular standard marker. The DNA fragments were separated by CHEF-DR® III Pulsed-Field Electrophoresis System (Bio-Rad Laboratories, Hercules, CA, USA).

Data analysis: Differences in resistance rate among the isolates from pigs and chickens were analyzed between Thailand and China by chi-squared analysis using SPSS software ver.17.0 (SPSS Inc., USA). Only data about resistance to AMP, CHL, NAL, SXT and TET were used in the analysis due to distribution of the data. The analysis was done separately for each antimicrobial agent. The fingerprint analysis was carried out using Bio-Numerics software version 7.6 (Applied Maths, Belgium). Calculation of similarity between PFGE fingerprint patterns was performed using the Dice coefficient similarity index with 1% optimization and 1% tolerance. Cluster analysis was performed using unweighted-pair group method with arithmetic means (UPGMA).

Results

Salmonella detection and serotypes: One hundred and six *Salmonella* isolates were recovered from the samples collected in Thailand. The percentage of *Salmonella* positive samples for pigs (35%, 36/200) was significantly higher than that for broilers (18%, 70/200) ($p < 0.05$). Of the 166 *Salmonella* isolates from Thailand and China, a total of 37 serotypes were identified (Table 1). The Thai isolates were composed of 18 serotypes, of which *S.* Rissen (34.0%) was the most common, followed by *S.* Give (21.7%) and *S.* Weltevreden (16.0%). The Chinese isolates consisted of 23 serotypes, of which *S.* Derby (21.7%) was the most common, followed by *S.* London (15.0%) and *S.* Give (10.0%).

Antimicrobial resistance of Salmonella isolates: The antimicrobial resistance percentages of *Salmonella* from Thailand and China are shown in Table 2. Differences in resistance proportion to antimicrobials were observed between the Thai isolates from pigs and broilers. All the Thai isolates from pigs were susceptible to norfloxacin and all the isolates from broilers were susceptible to amoxicillin/clavulanate. The pig isolates were most commonly resistant to ampicillin (70.0%), followed by tetracycline (50.0%), and trimethoprim/sulfamethoxazole (27.1%), while the broiler isolates exhibited a high frequency of resistance to nalidixic acid (69.4%), followed by ciprofloxacin (16.7%), and ampicillin (13.9%). In the pig isolates from China, all isolates were susceptible to amoxicillin/clavulanate and were commonly resistant to tetracycline (79.5%), followed by ampicillin (69.2%), chloramphenicol (56.4%) and trimethoprim/sulfamethoxazole (56.4%). The majority of broiler isolates from China exhibited resistance to ampicillin (85.7%), trimethoprim/sulfamethoxazole (85.7%) and tetracycline (85.7%). The resistance proportions to ampicillin, chloramphenicol, and nalidixic acid were significantly higher for both pig and broiler isolates from China than from Thailand ($p < 0.05$). A significantly higher resistance proportion to trimethoprim/sulfamethoxazole and tetracycline were also observed for the broiler isolates from China than from Thailand ($p < 0.05$). Overall, the Chinese isolates were more commonly found to show higher proportions of resistance to antimicrobials than the Thai isolates.

The antimicrobial resistance profiles of *Salmonella* isolates are shown in Table 3. The 166 *Salmonella* isolates displayed 32 different antimicrobial resistance profiles, with 38% (63/166) of the isolates being identified as multidrug resistance (MDR, resistance to 3 or more antimicrobial agents). In Thailand, 20.8% (22/106) of isolates were identified as MDR. The most frequent resistance profile was ampicillin/trimethoprim/sulfamethoxazole/tetracycline. In China, 68.3% (41/60) of isolates were identified as MDR, with the most common resistance pattern being ampicillin / chloramphenicol / trimethoprim / sulfamethoxazole / tetracycline.

Genetic diversity and antimicrobial resistance patterns (R-types) of Salmonella isolates: In order to investigate the relationship between the *Salmonella* isolates from Thailand and China, PFGE was performed in isolates of the serotypes commonly presented in both Thailand and China. Figure 1 shows the fingerprint patterns and R-types of 39 *Salmonella* isolates, comprising *S.* Typhimurium (n=13), *S.* Rissen (n=13) and *S.* Give (n=13). Based on 78% genetic similarity, the 39 *Salmonella* isolates were grouped into 9 genotypic clusters (A-I). The isolates of *S.* Typhimurium, *S.* Rissen and *S.* Give were distinctly grouped into 5 (A-E), 1 (F) and 3 (G-I) clusters, respectively. For *S.* Typhimurium and *S.* Give, the phylogenetic analysis revealed that the strains were clustered by geographical region. In contrast, all isolates of *S.* Rissen from Thailand and China were found to belong to the same cluster.

Most isolates in each cluster shared similar antimicrobial resistance patterns, except in the cluster F and G showing 4 diverse R-types. In terms of geographical location, distinct R-types were found for *S.* Typhimurium and *S.* Give isolated from Thailand and China. Conversely, some isolates of *S.* Rissen from Thailand and China had the same R-type such as isolates R9, R10, R11 (tetracycline) and R1, R2, R3 (amplicillin/tetracycline).

Discussion

In this study, *Salmonella* spp. was detected from pigs at a higher percentage than from broilers (35.0% versus 18.0%). For pigs, a similar percentage (34%) was reported in a previous study in northeastern Thailand (Sinwat et al., 2016). However, *Salmonella* positive proportions in this study were much higher than the studies in Sa Kaew province in eastern Thailand (35.0% versus 3%) and in two provinces in northern Thailand (35% versus 11.9%). Similarly, *Salmonella* spp. was detected from broilers at slaughterhouses at much higher percentages than the studies in Sa Kaew (18.0% versus 1.1%) and in Chiang Mai, a province located in the northern part of

Thailand (18.0% versus 3.2%) (Trongjit et al., 2017, Chotinun et al., 2014). These discrepancies may be due to the relatively high prevalence of *Salmonella* infections in pigs and broilers in the area of this study.

Table 1 Serotypes of *Salmonella* from pigs and broilers in Thailand and China

Salmonella Group	*Salmonella* Serotype	Thailand			China		
		Pigs (No, %)	Broilers (No, %)	Total (No, %)	Pigs (No, %)	Broilers (No, %)	Total (No, %)
A	*S*. Kiel					1, 4.8	1, 1.7
B	*S*. Derby				8, 20.5	5, 23.8	13, 21.7
	S. Typhimurium	8, 11.4		8, 7.5	5, 12.8		5, 8.3
	S. Stanley	7, 10		7, 6.6			
	S. Bredeney				1, 2.6		1, 1.7
	S. Fyris				1, 2.6		1, 1.7
	S. Kunduchi				1, 2.6		1, 1.7
	S. Indiana					1, 4.8	1, 1.7
	S. Travis					1, 4.8	1, 1.7
C	*S*. Rissen	36, 51.4		36, 34.0	2, 5.1	1, 4.8	3, 5.0
	S. Cremieu		2, 5.6	2, 1.9			
	S. Kentucky		2, 5.6	2, 1.9			
	S. Brunei	1, 1.4		1, 0.9			
	S. Bardo		1, 2.8	1, 0.9			
	S. Bazenheid		1, 2.8	1, 0.9			
	S. Chormedey		1, 2.8	1, 0.9			
	S. Doncaster					1, 4.8	1, 1.7
	S. Gyprus		1, 2.8	1, 0.9			
	S. Jerusalem					1, 4.8	1, 1.7
	S. Istanbul		1, 2.8	1, 0.9			
	S. Lichfield		1, 2.8	1, 0.9			
	S. Lomita				1, 2.6		1, 1.7
	S. Molade		1, 2.8	1, 0.9			
	S. Newport		1, 2.8	1, 0.9			
	S. Saintpaut		1, 2.8	1, 0.9			
	S. Wippra		1, 2.8	1, 0.9			
D	*S*. Enteritidis					1, 4.8	1, 1.7
E	*S*. Give	1, 1.4	22, 61.1	23, 21.7	3, 7.7	3, 14.3	6, 10.0
	S. Weltevreden	17, 24.3		17, 16.0		1, 4.8	1, 1.7
	S. London				7, 17.9	2, 9.5	9, 15.0
	S. Salamae				5, 12.8		5, 8.3
	S. Anatum				2, 5.1		2, 3.3
	S. Rideau				1, 2.6	1, 4.8	2, 3.3
	S. Calaber					1, 4.8	1, 1.7
	S. Newland					1, 4.8	1, 1.7
	S. Seegefeld				1, 2.6		1, 1.7
	S. Souza				1, 2.6		1, 1.7
Total		70, 100	36, 100	106, 100	39, 100	21, 100	60, 100

S. Rissen was the most common serotype among the isolates of Thai origin, particularly in the pig samples. This finding is similar to that of a previous study in which this serotype was found to be the most common in pork carcasses (Sanguankit et al., 2010). *S*. Rissen has been reported to be among the most common serotypes found in pigs and pork products in several Asian countries including South Korea, Vietnam, and Thailand (Lim et al., 2009; Thai et al., 2001; Hendriksen et al., 2008). In China, a recent study has shown that *S*. Rissen was the most predominant serotype in pigs at slaughterhouses in Shandong province (Zhao et al., 2017). Our results are different in that the most common serotype in pigs from China was *S*. Derby, and only 5% of *S*. Rissen were identified from pigs. *S*. Derby was also found to be the most common

among the Chinese isolates from broilers. However, this serotype was not found in the broilers in Thailand. In addition, the proportions of serotypes identified among the Thai and Chinese isolates are mostly different, except for *S.* Typhimurium in the pig isolates. A possible explanation for all these differences is the geographical locations of sampling sites.

Table 2 Antimicrobial resistance percentages of *Salmonella* from Thailand and China

		Antimicrobial resistant agants*								
Location	**Sample**	**AMP**	**AMC**	**CHL**	**CAZ**	**CIP**	**NAL**	**NOR**	**SXT**	**TET**
Thailand	Pig	49 (70.0%)[a]	1 (1.4%)	5 (7.1%)[a]	1 (1.4%)	2 (2.9%)	2 (2.9%)[a]	0 (0%)	19 (27.1%)	35 (50.0%)
	Broiler	5 (13.9%)[b]	0 (0%)	1 (2.8%)[b]	1 (2.8%)	6 (16.7%)	25 (69.4%)[b]	2 (5.6%)	2 (5.6%)[b]	1 (2.8%)[b]
China	Pig	27 (69.2%)[a]	0 (0%)	22 (56.4%)[a]	2 (5.1%)	4 (10.3%)	14 (35.9%)[a]	3 (7.7%)	22 (56.4%)	31 (79.5%)
	Broiler	18 (85.7%)[b]	3 (13.3%)	13 (61.9%)[b]	2 (9.5%)	6 (28.6%)	10 (47.6%)[b]	2 (9.5%)	18 (85.7%)[b]	18 (85.7%)[b]

*AMP: ampicillin, AMC: amoxycillin/clavulanate, CHL: chloramphenicol, CAZ: Ceftazidime, CIP: ciprofloxacin, NAL: nalidixic acid, NOR: norfloxacin, SXT: trimethoprim/sulfamethoxazole, TET: tetracycline

Table 3 Antimicrobial resistance profiles of *Salmonella* MDR from Thailand and China

	Thailand			China		
Antimicrobial resistant profile	Pig **(No, %)**	Broiler **(No, %)**	Total **(No, %)**	Pig **(No, %)**	Broiler **(No, %)**	Total **(No, %)**
AMP-CAZ-NAL	1, 5.0		1, 4.5			
AMP-NAL-SXT					1, 5.6	1, 2.4
AMP-SXT-TET	13, 65.0		13, 59.1	1, 4.3	2, 11.1	3, 7.3
CHL-SXT-TET	2, 10.0		2, 9.1			
AMP-AMC-SXT-TET	1, 5.0		1, 4.5			
AMP-CHL-SXT-TET	2, 10.0		2, 9.1	10, 43.5	6, 33.3	16, 39.0
AMP-CIP-NAL-TET	1, 5.0		1, 4.5			
CHL-NAL-SXT-TET				1, 4.3		1, 2.4
CAZ--CIP-NAL-NOR		1, 50.0	1, 4.5			
AMP-CHL-NAL-SXT-TET				6, 26.1	1, 5.6	7, 17.1
AMP-AMC-CHL-SXT-TET					1, 5.6	1, 2.4
AMP-CAZ-NAL-SXT-TET				1, 4.3	1, 5.6	2, 4.9
AMP-CIP-NAL-SXT-TET					1, 5.6	1, 2.4
AMP-CIP-NAL-NOR-TET		1, 50.0	1, 4.5			
AMP-CHL-CIP-NOR-SXT-TET				1, 4.3		1, 2.4
AMP-CHL-CIP-NAL-SXT-TET					3, 16.7	3, 7.3
AMP-CHL-CAZ-CIP-NAL-TET				1, 4.3		1, 2.4
AMP-CHL-CIP-NAL-NOR-SXT-TET				2, 8.7		2,4.9
AMP-AMC-CHL-CIP-NAL-NOR-SXT-TET					1, 5.6	1, 2.4
AMP-CHL-CAZ-CIP-NAL-NOR-SXT-TET					1, 5.6	1, 2.4
Total	20, 100	2, 100	22, 100	23, 100	18, 100	41, 100

In Thailand, the highest resistance proportion was observed for ampicillin, followed by tetracycline. This finding is similar to that in China where bacteria were most commonly resistant to ampicillin and tetracycline. This may be attributed to the global usage of ampicillin and tetracycline in food-animals against bacterial infections for long periods, causing selective pressure. Chloramphenicol has already been banned in food-animal productions for more than 10 years in most countries including Thailand and China. However, *Salmonella* isolates of Thai and Chinses origins were found to have resistance to chloramphenicol, particularly the Chinese isolates. Resistance to chloramphenicol is related to the presence of chloramphenicol resistance gene (*cml*A) located in gene cassettes in class 1 integrons. Thus, *Salmonella* carrying class 1 integrons may display chloramphenicol resistance phenotype (Chuanchuen et al., 2009, Shousha et al., 2015, Prasertsee et al., 2016). Another reason mentioned in a previous study is that the use of related classes of antibiotics such as florfenicol, commonly used in livestock production,

attributes to co-selective pressure (Pornsukarom et al., 2015). High resistance proportions to nalidixic acid (first generation quinolones antibiotics) were detected in pigs from China (35.9%) and in broilers from both Thailand (69.4%) and China (47.6%). Quinolones are commonly used for the treatment of multi-drug resistant salmonellosis in humans and animals (Dalhoff, 2012). Thus, increasing quinolone resistance means increased difficulty in controlling bacterial infections caused by *Salmonella* spp. (Pribul et al., 2017).

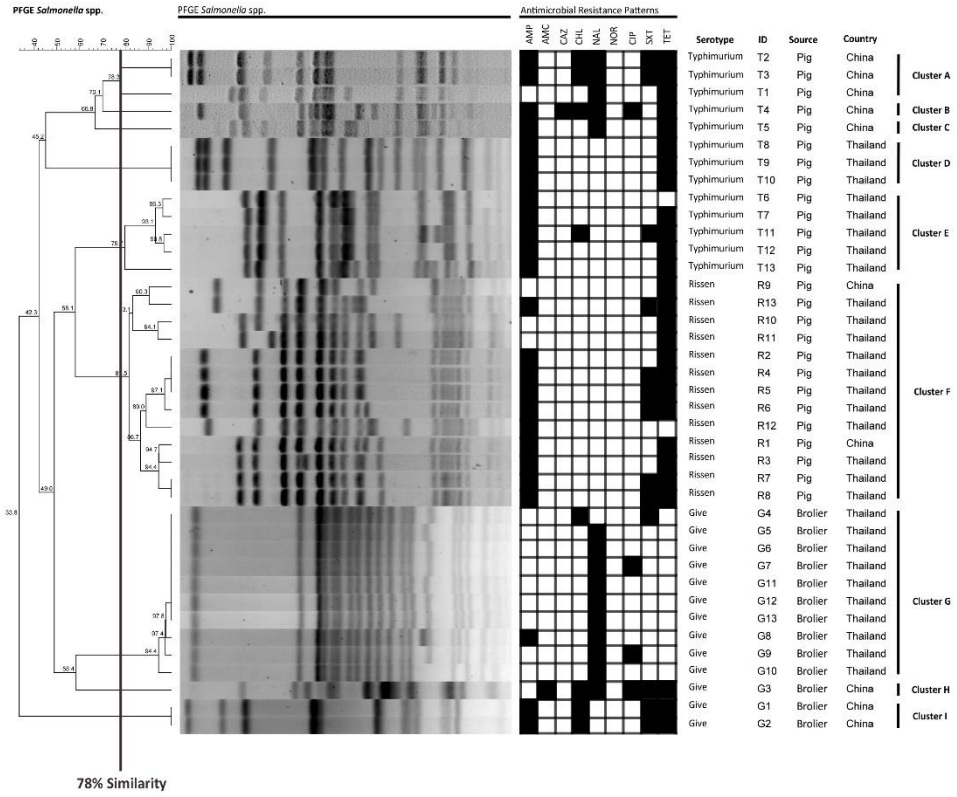

Figure 1 Dendrogram of 39 PFGE-*Xba*I profiles of *Salmonella* spp. isolated from broilers and pigs at slaughterhouses in Thailand and China (■ = resistance, □ = susceptibility)

The serotyping results demonstrated that *S.* Typhimurium, *S.* Rissen, *S.* Give and *S.* Weltevreden were present in both Thailand and China. Pulsed-Field Gel Electrophoresis (PFGE) has been used as an epidemiological tool to identify the genetic relatedness of *Salmonella* across different geographical regions (Zheng et al., 2014). In this study, *S.* Typhimurium, *S.* Rissen and *S.* Give were genetically analyzed by PFGE in order to investigate the relationship between the *Salmonella* isolates from Thailand and China. The pulsotypes and also R-types of *S.* Typhimurium and *S.* Give isolates did not show a relationship between these two different geographical regions. However, these findings are different from those found in *S.* Rissen. The pulsotype of *Salmonella* isolates revealed the genetic relatedness between *S.* Rissen from Thailand and China. This finding differs from a previous study that demonstrated no clonal relatedness between *S.* Rissen isolates from Thailand and United States (Pornsukarom et al., 2015). It could be due to a wider spectrum of clonal diversity among *S.* Rissen in the US than in Thailand and China. However, the epidemiological link between *S.* Rissen in Thailand and China is possible and calls for further investigations.

In conclusion, this is the first study that compares molecular epidemiology and antimicrobial resistance of *Salmonella* spp. between Thailand and China. It indicated that *S.* Rissen was the most important serotype for implementation of active surveillance systems to monitor and control cross-circulation and spread of AMR between Thailand and China through various means such as bilateral trade and tourism.

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References

Castanon JI 2007. History of the use of antibiotic as growth promoters in European poultry feeds. Poult Sci. 86(11): 2466-2471.

Centers for Disease Control and Prevention (CDC) 2017. "Standard operating procedure for PulseNet PFGE of *Escherichia coli* O157:H7, *Escherichia coli* non-O157 (STEC), *Salmonella* serotypes, *Shigella sonnei* and *Shigella flexneri*" [Online]. Available at: https://www.cdc.gov/pulsenet/pdf/ecoli-shigella-salmonella-pfge-protocol-508c.pdf Accessed December 2017.

Chotinun S, Rojanasthien S, Unger F, Suwan M, Tadee P and Patchanee P 2014. An integrative approach to enhancing small-scale poultry slaughterhouses by addressing regulations and food safety in northern -Thailand. Infect Dis Poverty. 3(1): 46.

Chuanchuen R and Padungtod P 2009. Antimicrobial resistance genes in *Salmonella enterica* isolates from poultry and swine in Thailand. J Vet Med Sci. 71(10): 1349-1355.

Clemente L, Manageiro V, Jones-Dias D, Correia I, Themudo P, Albuquerque T, Geraldes M, Matos F, Almendra C, Ferreira E and Caniça M 2015. Antimicrobial susceptibility and oxymino-β-lactam resistance mechanisms in *Salmonella enterica* and *Escherichia coli* isolates from different animal sources. Res Microbiol. 166(7): 574-583.

Clinical and Laboratory Standards Institute (CLSI) 2017. "Performance Standards for Antimicrobial Susceptibility Testing 27TH Edition" [Online]. Available at: https://clsi.org/media/1469/m100s27_sample.pdf Accessed January 2017.

Dalhoff A 2012. Resistance surveillance studies: a multifaceted problem—the fluoroquinolone example. Infection. 40(3): 239-262.

Guerra B, Fischer J and Helmuth R 2014. An emerging public health problem: acquired carbapenemase-producing microorganisms are present in foodproducing animals, their environment, companion animals and wild birds. Vet Microbiol. 171(3-4): 290-297.

Hendriksen RS, Bangtrakulnonth A, Pulsrikarn C, Pornreongwong S, Hasman H, Song SW and Aarestrup FM 2008. Antimicrobial resistance and molecular epidemiology of *Salmonella* Rissen from animals, food products, and patients in Thailand and Denmark. Foodborne Pathog Dis. 5(5): 605-619.

Hendriksen RS, Vieira AR, Karlsmose S, Lo Fo Wong DM, Jensen AB, Wegener HC and Aarestrup FM 2011. Global monitoring of *Salmonella* serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: results of quality assured laboratories from 2001 to 2007. Foodborne Pathog Dis. 8(8): 887-900.

International Organization for Standardization (ISO) 2017. "Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Part 1: Detection of *Salmonella* spp. " [Online]. Available at: https://www.iso.org/standard/56712.html Accessed Febrary 2017.

Landers TF, Cohen B, Wittum TE and Larson EL 2012. A Review of Antibiotic Use in food animals: perspective, policy, and potential. Public Health Rep. 127(1): 4-22.

Lim SK, Lee HS, Nam HM, Jung SC, Koh HB and Roh IS 2009. Antimicrobial resistance and phage types of *Salmonella* isolates from healthy and diarrheic pigs in Korea. Foodborne Pathog Dis. 6(8): 981-987.

Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, Jones TF, Fazil A, Hoekstra RM and International Collaboration on Enteric Disease 'Burden of Illness' Studies 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. Clin Infect Dis, 50(6): 882-889.

Manarungsan S 2009. "Thailand-China cooperation in trade, investment and official development assistance." [Online]. Available at: http://www.ide.go.jp/library/English/Publish/Download/Brc/pdf/01_thailandandchina.pdf Accessed December 2009.

Pornruangwong S, Sriyapai T, Pulsrikarn C, Sawanpanyalert P, Boonmar S and Bangtrakulnonth A 2008. The epidemiological relationship between *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar 4,[5],12:i:- isolates from humans and swine in Thailand. Southeast Asian J Trop Med Public Health. 39(2): 288-296.

Pornsukarom S, Patchanee P, Erdman M, Cray PF, Wittum T, Lee J and Gebreyes WA 2015. Comparative phenotypic and genotypic analyses of *Salmonella* Rissen that originated from food animals in Thailand and United States. Zoonoses Public Health. 62(2): 151-158.

Prasertsee T, Khantaprab N, Yamsakul P, Santiyanont P, Choksajjawatee N and Patchnee P 2016. Repetitive sequence-based PCR fingerprinting and the relationship of antimicrobial-resistance characteristics and corresponding genes among *Salmonella* strains from pig production. Asian Pac J Trop Dis. 6(5): 390-395.

Pribul BR, Festivo ML, Rodrigues MS, Costa RG, Rodrigues EC, de Souza MM and Rodrigues DD 2017. Characteristics of quinolone resistance in *Salmonella* spp. isolates from the food chain in Brazil. Front Microbiol. 8: 299.

Read AF and Woods RJ 2014. Antibiotic resistance management. Evol Med Public Health. 2014(1): 147.

Sanguankiat A, Pinthong R, Padungtod P, Baumann MP, Zessin KH, Srikitjakarn L and Fries R 2010. A cross-sectional study of *Salmonella* in pork products in Chiang Mai, Thailand. Foodborne Pathog Dis. 7(8): 873-878.

Shousha A, Awaiwanont N, Sofka D, Smulders FJ, Paulsen P, Szostak MP, Humphrey T and Hilbert F 2015. Bacteriophages isolated from chicken meat and the horizontal transfer of antimicrobial resistance genes. Appl Environ Microbiol. 81(14): 4600-4606.

Sinwat N, Angkittitrakul S, Coulson KF, Pilapil FM, Meunsene D and Chuanchuen R 2016. High prevalence and molecular characteristics of multidrug-resistant *Salmonella* in pigs, pork and humans in Thailand and Laos provinces. J Med Microbiol. 65(10): 1182-1193.

Sirichote P, Hasman H, Pulsrikarn C, Schonheyder HC, Samulioniene J, Pornruangmong S, Bangtrakulnonth A, Aarestrup FM and Hendriksen RS 2010. Molecular characterization of extended-spectrum cephalosporinase-producing *Salmonella enterica* serovar Choleraesuis isolates from patients in Thailand and Denmark. J Clin Microbiol. 48(3): 883-888.

Thailand Ministry of Commerce. "Exports of Thailand Classified by country (Jan. - Mar. 2017) Country: China&HK". [Online]. Available at: http://www.ops3.moc.go.th/infor/hs/export/export_country_hsrank/report.asp Accessed June 2017.

Thai TH and Yamaguchi R 2012. Molecular characterization of antibiotic-resistant Salmonella isolates from retail meat from markets in Northern Vietnam. J Food Prot. 75(9): 1709-1714.

Trongjit S, Angkititrakul S, Tuttle RE, Poungseree J, Padungtod P and Chuanchuen R 2017. Prevalence and antimicrobial resistance in *Salmonella enterica* isolated from broiler chickens, pigs and meat products in Thailand-Cambodia border provinces. Microbiol Immunol. 61(1): 23-33.

Ventola CL 2015. The Antibiotic Resistance Crisis: Part 1: Causes and Threats. J Clin Pharm Ther. 40(4): 277-283.

Witte W 2000. Selective pressure by antibiotic use in livestock. Int J Antimicrob Agents. 16 Suppl 1:S19-S24.

Zhao X, Ye C, Chang W and Sun S 2017. Serotype Distribution, Antimicrobial Resistance, and Class 1 Integrons Profiles of *Salmonella* from Animals in Slaughterhouses in Shandong Province, China. 8: 1049.

Zheng J, Pettengill J, Strain E, Allard MW, Ahmed R, Zhao S and Brown EW 2014. Genetic diversity and evolution of *Salmonella enterica* serovar Enteritidis strains with different phage types. J Clin Microbiol. 52(5): 1490-1500.

บทคัดย่อ

ระบาดวิทยาทางโมเลกุลและการดื้อต่อสารต้านจุลชีพของเชื้อซัลโมเนลลาที่แยกได้จากไก่และสุกรที่โรงฆ่าสัตว์ของประเทศไทยและประเทศสาธารณรัฐประชาชนจีน

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วัตถุประสงค์ของการศึกษาครั้งนี้ เพื่อเปรียบเทียบรูปแบบการดื้อต่อสารต้านจุลชีพและ DNA restriction pattern โดยใช้วิธี Pulsed-Field Gel Electrophoresis (PFGE) ของเชื้อ *Salmonella* spp. ที่แยกได้จากไก่และสุกรที่โรงฆ่าสัตว์ในประเทศไทยและประเทศสาธารณรัฐประชาชนจีน จำนวนตัวอย่างทั้งหมด 166 ตัวอย่าง โดยสุ่มเก็บจากไก่และสุกรที่โรงฆ่าสัตว์ในจังหวัดขอนแก่น ประเทศไทย (n=106) ระหว่างเดือนกุมภาพันธ์ถึงสิงหาคม 2560 และเมืองฉงชิ่ง ประเทศสาธารณรัฐประชาชนจีน (n=60) ระหว่างเดือนมีนาคมถึงตุลาคม 2558 การทดสอบการดื้อต่อสารต้านจุลชีพจำนวน 9 ชนิดด้วยวิธี Disk diffusion method การเปรียบเทียบความหลายหลายทางพันธุกรรมของเชื้อซัลโมเนลลาด้วยวิธี PFGE ด้วยเอนไซม์ *Xba*I การศึกษาพบเชื้อซัลโมเนลลา 37 ซีโรไทป์จาก 166 ตัวอย่าง เชื้อที่แยกจากประเทศไทยพบ 18 ซีโรไทป์ โดยเป็น *S.* Rissen สูงสุด (34%) ส่วนประเทศจีนพบ 23 ซีโรไทป์ โดยเป็น *S.* Derby มากที่สุด (22%) พบการดื้อต่อสารต้านจุลชีพทั้งหมดร้อยละ 38 (63/166) โดยเป็นเชื้อที่ดื้อต่อสารต้านจุลชีพสามชนิดขึ้นไปของประเทศไทยและประเทศจีนจำนวน 20.8% (22/106) และ 68.3% (41/60) ตามลำดับ อย่างไรก็ดี ไม่พบความสัมพันธ์ของ *S.* Typhimurium และ *S.* Give ที่แยกได้จากประเทศไทยและประเทศจีน แต่ซีโรไทป์ *S.* Rissen พบว่ามีความสัมพันธ์กันมากกว่าร้อยละ 90 ดังนั้น การเฝ้าระวังเชื้อซัลโมเนลลาอย่างเป็นระบบ โดยเฉพาะ *S.* Rissen และการใช้สารต้านจุลชีพอย่างเหมาะสมในสัตว์บริโภคทั้งประเทศไทยและประเทศจีนจะช่วยลดการแพร่กระจายและการดื้อต่อสารต้านจุลชีพของเชื้อนี้ได้

คำสำคัญ: ซัลโมเนลลา การดื้อต่อสารต้านจุลชีพ PFGE ประเทศไทย ประเทศสาธารณรัฐประชาชนจีน

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