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Multidrug Resistance and Serotype Distribution of *Salmonella Enterica* Isolated from Homemade Recipe Fermented Ground Pork (Nham) in Northeastern Thailand

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Abstract

Salmonellosis is caused by a thousand serotypes of *Salmonella enterica*. The sour taste inherent to Nham leads people believe that this fermented ground pork dish is safe from pathogenic microorganisms. The aim of this study was to evaluate the prevalence, serotype, drug susceptibility, and antimicrobial resistance (AMR) genes of *Salmonella* spp. in homemade recipes of Nham. There were 52 samples from different Nham makers in 3 northeastern provinces of Thailand collected between August and November 2019. Further, 30 Salmonella isolates (57.7 %) and 14 different serovars were identified: *S*. Rissen (23.3 %) was the most prevalent, followed by *S*. Typhimurium (16.7 %), *S*. Give and *S*. Virchow (10 % each), and *S*. Agona and *S*. Kouka (6.7 % each). All isolates carried AMR genes but 7 (23.3 %) were antibiotic susceptible and 23 (76.7 %) borne a resistance phenotype. The Salmonella isolates were resistant to tetracycline (63 %), sulfamethoxazole/trimethoprim (36.7 %), streptomycin (33.3 %), nalidixic acid (30 %), cefotaxime (16.7 %), and enrofloxacin (3.3 %). Among the 23 AMR genes in our analysis, there were *gyrB* (100 %), *tetA* (93.3 %), *aadA* (93.3 %), *sul1*, *sul2*, *sul3* (23.3 - 33.3 %), *dfrA12* (16.7 %), *qnrS*, (6.7 %), and *mcr6* (6.7 %). Two strains had the *mcr6* gene but were susceptible to colistin. Our findings suggest that naturally occurring lactic acid bacteria in the Nham products are insufficient to inhibit Salmonella contamination of this pork-based food.

Keywords: Antimicrobial resistance, Gene, Nham, Pork, *Salmonella*

Introduction

Salmonellosis is considered as a zoonotic disease and the route of infection is often linked to pork consumption [1]. While systematic public health monitoring is on-going, a local survey is necessary to assess specific factors and the risks of infection in the food chain. In Thailand, the prevalence of pigs carrying Salmonella has been reported to be around 35 % [2], but Salmonella contamination rates in pork were 82 - 86 % [3,4]. This huge detection gap is probably due to cross-carcass contamination during cutting at lairages or handling at retail markets. A homemade recipe for fermented ground pork (Nham) consists of minced pork meat mixed with thinly sliced boiled pig skin, garlic, salt, sugar, cooked rice, and incubated at room temperature for natural acidic fermentation. Nham is a traditional food and is preferentially consumed raw in the northern and northeastern regions of Thailand. Its sour taste leads people to believe that Nham is safe from pathogenic microorganisms [5]. However, this belief cannot be guaranteed as seen by the high incidences of Salmonella food poisoning in northeastern Thailand. Homemade Nham recipes vary in their ingredients, which natural lactic acid-producing bacteria are used for the fermentation, and the critical temperatures used in each step. To ensure safety of this product, it is necessary to survey the quality regularly. The aim of this study was to investigate the prevalence, serotypes, antimicrobial susceptibility, and the antimicrobial resistance genes

present in the *Salmonella* spp. isolated from homemade Nham prepared in 3 northeastern provinces of Thailand.

Materials and methods

Sample size calculation

Sample size was calculated by using the "Estimate Percentage" function [6]. Considering "85 %" to be the approximate rate of Salmonella contamination in pork samples in Thailand [4], this value was used as the expected prevalence. The "e" and "Z1-α/2" values were selected as 10 % and 0.95, respectively, as the necessary feature parameters. A sample size of at least 49 was indicated.

Sample collection

Homemade Nham samples were randomly collected at local retail shops representing 52 different Nham makers in the Khon Kaen (n = 20), Kalasin (n = 16), and Roi-Ed (n = 16) provinces between August and November 2019. The venders' consents did not include the disclosure of their names. All samples were kept in a sterile plastic bag individually, then transported to the laboratory within 10 h. The Salmonella culturing was performed at the Veterinary Diagnostic Laboratory of Research, Faculty of Veterinary Medicine, Khon Kaen University, Thailand.

Salmonella isolation and identification

For each brand, 3 to 5 pieces per batch of Nham were removed and each corresponding package was opened to check for pH of below 5 before bacterial culturing took place. If the pH was not yet less than 5, then the other pieces were allowed to ferment until they reached this acidic pH. The pH value of Nham was determined by homogenizing 10 g of Nham in 100 mL of sterile distilled water and then using a pH meter (Merk, Coulter). The isolation of *Salmonella* spp. was performed according to standard methods (ISO 6579:2002/Amendment 1:2017, Annex D). In brief, 25 g of Nham was added into 225 mL of Buffered Peptone Water (BPW, Oxoid, United Kingdom), mixed, and incubated at 37 °C for 18 - 24 h. The inoculated BPW was then transferred to Modified Semi-Solid Rappaport Vassiliadis (MSRV) medium (Oxoid, United Kingdom) using 3 loops; each loop was inoculated at the peripheral area on the MSRV plate and then incubated at 42 °C for 18 - 24 h. Positive colonies in the MSRV media were transferred to xylose lysine deoxycholate agar (XLD, Oxoid, United Kingdom), and then incubated at 37 °C for 18 - 24 h. Three Salmonella-positive colonies were chosen to be transferred into triple sugar iron agar (TSI, Oxoid, United Kingdom) and motility indole lysine medium (MIL, Himedia, India) then incubated at 37 °C for 18 - 24 h. Only typical Salmonella biochemistry in the TSI and MIL tests were selected for further grouping and serotyping.

Salmonella grouping and serotyping

A Kauffman-White classification scheme was used for grouping and serotyping the Salmonella isolates [7]. All Salmonella isolates were serotyped at the Regional Medical Sciences Center 7, Ministry of Public Health, Khon Kaen province, Thailand. The slide agglutination test against O and H antigens utilizing commercial antisera (S & A. Reagent Laboratory LMT, Bangkok, Thailand) was used to group the Salmonella isolates following the manufacturing's instruction.

Antimicrobial susceptibility testing

The disc diffusion method was employed, and this procedure was in compliance with the Clinical and Laboratory Standards Institute (CLSI) [8]. Seven antimicrobial agents were used for susceptibility, including cefotaxime 30 µg (CTX), nalidixic acid 30 µg (NAL), enrofloxacin 5 µg (ENR), streptomycin 10 µg (STR), sulfamethoxazole/trimethoprim 25 µg (SXT), and tetracycline 30 µg (TET) (Oxoid, United Kingdom). The minimum inhibitory concentration (MIC) and the micro-broth dilution method were used to determine colistin susceptibility [9]. The standard *Escherichia coli* ATCC® 25922 was included as a quality control. The result of the antimicrobial resistance was determined by the diameter of the inhibition zone and the MIC breakpoints according to CLSI [8] and EUCAST [10].

Identification of antimicrobial resistance genes

Chromosomal DNA was extracted using the GF-1 nucleic acid extraction kit (Vivantis, Selangor Darul Ehsan, Malaysia). PCR primers used in this study are listed in **Table 1**. All PCR amplifications were performed using 2X Vired PCR Master Mix (Vivantis, Selangor Darul Ehsan, Malaysia). The PCRs were performed in a 20 µL volume containing 10 ng DNA, 0.5 µM each of forward and reverse primers, and 10 µL 2X Vired Taq Master Mix (Vivantis, Selangor Darul Ehsan, Malaysia). For nucleotide sequencing analysis, PCR amplicons were purified using the GF-1 AmbiClean kit gel and PCR (Vivantis; Selangor Darul Ehsan) and then submitted for further analysis at the First Base Company, Malaysia. The

DNA sequences obtained were compared to the Genbank database using the blast algorithm available on the National Center for Biotechnology Information website [11].

All nalidixic acid-resistant Salmonella strains were further examined for mutations in the quinolone resistance determinant region (QRDR) of *gyrB* and *parC* [12] using PCR and DNA sequencing. The *gyrB* and *parC* of 2 standard nalidixic acid-susceptible strains were included as negative controls. The presence of plasmid-mediated quinolone resistance (PMQR) on the *qnrA*, *qnrB*, and *qnrS* genes was determined in all Salmonella isolates as described previously [13]. The *E. coli qnr*-positive strains served as positive controls [14]. Multiplex PCR was employed to screen 8 colistin resistance genes (*mcr1-8*), then confirmed the positive samples by single PCR under the same condition [15-17].

Table 1 Antimicrobial resistance genes, primer sequences, amplicon sizes, and annealing temperatures utilized in our study.

No.	Gene	Primer	Primer sequences	Tm (°C)	Size (bp)	Reference
1	*tetA*	tetA F	GCTACATCCTGCTTGCCTTC	50	210	[18]
		tetA R	CATAGATCGCCGTGAAGAGG			
2	*tetB*	tetB F	CAGTGCTGTTGTTGTCATTAA	50	571	[12]
		tetB R	GCTTGGAATACTGAGTGTAA			
3	*tetC*	tetC F	CTTGAGAGCCTTCAACCCAG	55	418	[19]
		tetC R	ATGGTCGTCATCTACCTGCC			
4	*qnrA*	qnrA F	ATTTCTCACGCCAGGATTTG	55	516	[20]
		qnrA R	GATCGGCAAAGGTTAGGTCA			
5	*qnrB*	qnrB F	TCGGCTGTCAGTTCTATGATCG	57	496	[13]
		qnrB R	TCCATGAGCAACGATGCCT			
6	*qnrS*	qnrS-F	TGATCTCACCTTCACCGCTTG	58	566	[13]
		qnrS-R	GAATCAGTTCTTGCTGCCAGG			
7	*dfrA12*	dfrA12-F	TTAGCCGTTTCGACGCGCAT	55	498	[21]
		dfrA12-R	ATGAACTCGGAATCAGTACGC			
8	*aadA*	aadA F	TGATTTGCTGGTTACGGTGAC	56	284	[22]
		aadA R	CGCTATGTTCTCTTGCTTTTG			
9	*sul1*	sul1-F	TGGTGACGGTGTTCGGCATTC	55	790	[12]
		sul1-R	GCGAGGGTTTCCGAGAAGGTG			
10	*sul2*	sul2-F	CCTGTTTCGTCCGACACAGA	53	435	[12]
		sul2-R	GAAGCGCAGCCGCAATTCAT			
11	*sul3*	sul3-F	ATGAGCAAGATTTTTGGAATCGTA	53	792	[12]
		sul3-R	A CTAACCTAGGGCTTTGGATATTT			
14	*gyrB*	gyrB F	CTGCGCTATCACAGCATCAT	56	219	[12]
		gyrB R	CGCGATGGAAATCTGGTACT			
15	*parC*	parC F	CTATGCGATGTCAGAGCTGG	59	262	[23]
		parC R	TAACAGCAGCTCGGCGTATT			
16	*mcr1*	mcr1F	TTGCAAATTCACGCCAGTG	62	383	[17]
		mcr1R	CTTTGACTTTGTCCGCGGTG			
17	*mcr2*	mcr2 F	CAAGTGTGTTGGTCGCAGTT	62	715	
		mcr2 R	TCTAGCCCGACAAGCATACC			
18	*mcr3*	mcr3 F	CTGAACTGGCGTGGAGTTCT	62	1350	
		mcr3 R	ATCATCCGGTGCAAACTGGT			
19	*mcr4*	mcr4 F	TCACTTTCATCACTGCGTTG	62	1116	[16] [15]
		mcr4 R	TTGGTCCATGACTACCAATG			
20	*mcr5*	mcr5 F	ATGCGGTTGTCTGCATTTATC	62	1641	
		mcr5 R	TCATTGTGGTTGTCCTTTTCTG			
21	*mcr6*	mcr6 F	GTCCGGTCAATCCCTATCTGT	62	1022	
		mcr6 R	ATCACGGGATTGACATAGCTAC			
22	*mcr7*	mcr7 F	TGCTCAAGCCCTTCTTTTCGT	62	892	
		mcr7 R	TTCATCTGCGCCACCTCGT			
23	*mcr8*	mcr8 F	GCGGGTAACCAACCCCTATC	62	265	
		mcr8 R	TGCCGGCATATCATCTGTGG			

Legend: F-Forward, R-Reverse

Results

The Nham samples had an average pH of 4.25 ± 0.25 (median value of 4.21; lowest 3.68 to highest 4.97). A total of 30 Salmonella isolates were detected from 52 samples. The overall prevalence of Salmonella in Nham was 57.7 %. Nham collected from the Khon Kaen (60 %, 12/20), Kalasin (62.5 %, 10/16), and Roi Et (50 %, 8/16) provinces were Salmonella-isolate positive. There were 14 different serovars of Salmonella identified in this study. The Salmonella serovars identified in Nham in descending frequency were *S.* Rissen (23.3 %, 7/30), *S.* Typhimurium (16.7 %, 5/30), *S.* Give, and *S.* Virchow (10 %, 3/30 each), *S.* Agona, and *S.* Kouka (6.7 %, 2/30 each). There was one isolate in each of the following serovars found in each Nham sample, *S.* Brandenburg, *S.* Derby, *S.* Hvittingfoss, *S.* Idiken, *S.* London, *S.* Paratyphi B, *S.* Senftenberg, and *S.* Regent (3.3 %, 1/30 each) (**Figure 1**).

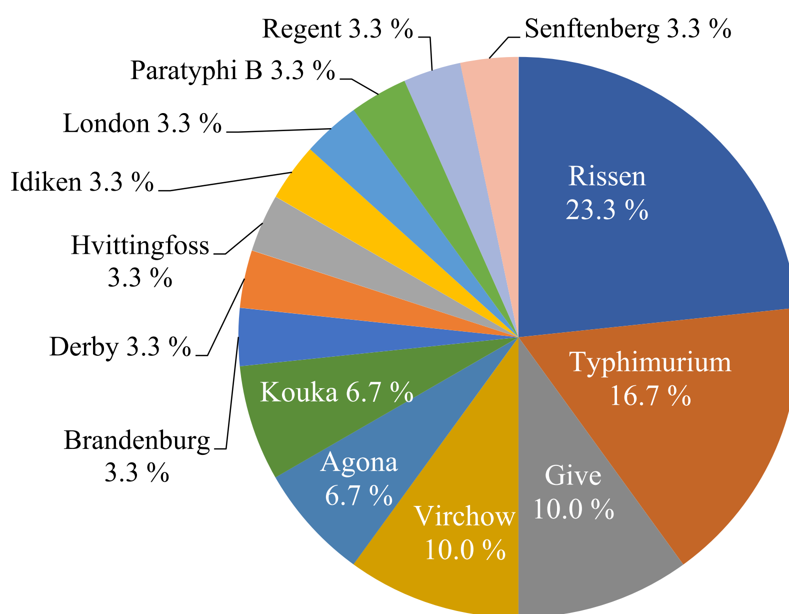

Figure 1 Serotypes of Salmonella isolated from Nham collected from the Khon Kaen, Kalasin, and Roi Et provinces, northeastern Thailand (n = 30).

Salmonella isolates were resistant to tetracycline (63.3 %), sulfamethoxazole/trimethoprim (63.7 %), streptomycin (33.3 %), nalidixic acid (30 %), cefotaxime (16.7 %), and enrofloxacin (3.3 %). In contrast, all 30 isolates were susceptible to colistin (**Figure 2**). Among the 30 Salmonella isolates, 7 (23.3 %) were antibiotic susceptible, whereas 23 (76.7 %) were resistant to antibiotics**.** Of these resistant isolates, 6 isolates resisted one drug, another 7 isolates were resistant to 2 classes of antibiotics, and the last 10 isolates borne multiple drug resistance (MDR) (**Table 2**). There was only one isolate belonging to serovar *S.* Kouka that resisted enrofloxacin (3.3 %) and 5 isolates that were cefotaxime resistant (16.7 %) (**Table 2**). The multidrug-resistant isolates, which resisted at least 3 drugs, belonged to serovars *S.* Agona, *S.* Give, *S.* Hvittingfoss, *S.* Kouka, *S.* London, *S.* Regent, *S.* Rissen, and *S.* Typhimurium (**Table 2**).

Conventional PCR was used to analyze the 23 AMR genes associated with colistin, quinolone, streptomycin, tetracycline, sulfamethoxazole, trimethoprim, and colistin antibiotics. **Table 2** presents detailed results of the AMR genes carried by each isolate. The highest prevalence of AMR genes was *gyrB* (100.0 %) followed by *tetA* and *aadA* (93.3 %). All of the sulfonamide resistance genes (*sul1*, *sul2*, *sul3*) were detected with 23.3 to 30.0 % prevalence, but the trimethoprim resistance gene, *dfrA12*, was found to have only 16.67 % prevalence. Only one plasmid-mediated quinolone resistance gene, *qnrS,* was found (6.7 %) in the isolates examined, which was detected in *S.* Typhimurium**.** One colistin resistance gene, *mcr*6, was detected (6.7 %) in our analysis (**Figure 3**), as well. Serotypes carrying *mcr6* were *S.* Brandenburg and *S.* Rissen (**Table 2**).

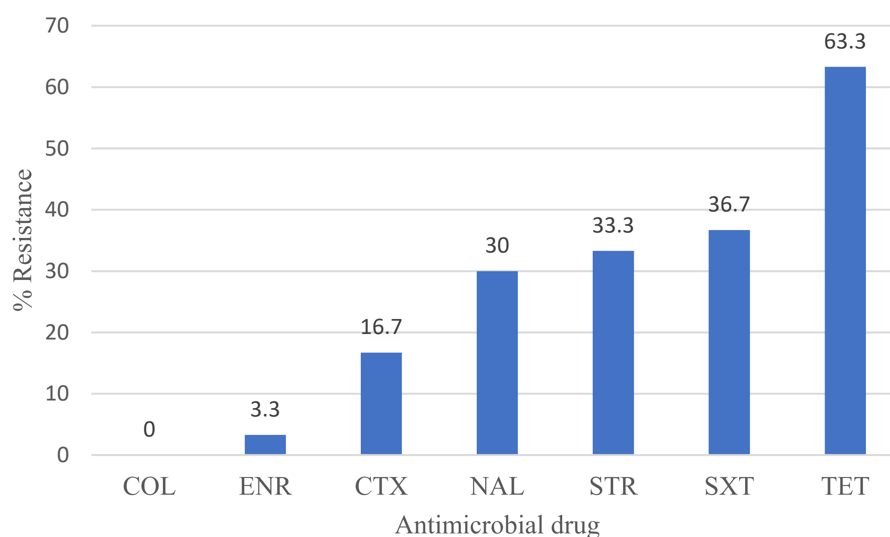

Figure 2 The proportion of the 30 antimicrobial-resistant Salmonella isolates against the 7 classes of antimicrobial drugs.
Legend: CTX-cefotaxime, NAL-nalidixic acid, ENR-enrofloxacin, STR-streptomycin, SXT-sulfamethoxazole/ trimethoprim, TET-tetracycline, COL-colistin (for this drug, determined by MIC)

Table 2 Antimicrobial resistant patterns and AMR gene profiles of the 30 Salmonella isolates.

No. sample	Serovar	Resistant pattern (MIC and/or agar disc diffusion)	Resistance gene
1. NH-7	Agona	susceptible	*tetA, aadA, gyrB*[a]
2. NH-21	Agona	STR- SXT- TET	*tetA, aadA, sul3, gyrB*[a]
3. NH-15	Brandenburg	susceptible	*tetA, tetB, sul1, aadA, gyrB*[a], *parC*[d], *mcr6*
4. NH-2	Derby	SXT- TET	*tetA, dfrA12, aadA, sul1, sul3, gyrB*[a], *parC*[b]
5. NH-28	Give	CTX-NAL-STR-SXT-TET	*tetA, dfrA12, addA, sul1, gyrB*[a]
6. NH-31	Give	NAL	*tetA, addA, gyrB*[b]
7. NH-35	Give	NAL	*tetA, gyrB*[a], *parC*[d,e,f]
8. NH-9	Hvittingfoss	NAL- STR- SXT	*tetA, aadA, sul1, gyrB*[a], *parC*[b]
9. NH-10	Idiken	SXT-TET	*tetA, sul3, aadA, gyrB*[a], *parC*[d]
10. NH-24	Kouka	STR- TET	*tetA, aadA, sul3, gyrB*[a], *parC*[d,e,f]
11. NH-27	Kouka	CTX-ENR-NAL-STR-SXT	*tetA, addA, sul2, gyrB*[a]
12. NH-22	London	CTX- SXT- TET	*tetA, addA, gyrB*[a]
13. NH-32	Paratyphi B	susceptible	*tetA, addA, gyrB*[a]
14. NH-33	Regent	NAL- SXT- TET	*tetA, dfrA12, addA, sul3, qnrS, gyrB*[b], *parC*[b]
15. NH-12	Rissen	TET	*tetA, sul3, aadA, gyrB*[a], *parC*[e]
16. NH-13	Rissen	STR- SXT- TET	*tetA, tetB, sul1, sul2, aadA, gyrB*[a], *parC*[b]
17. NH-19	Rissen	STR- TET	*tetA, tetB, aadA, sul1, gyrB*[a], *mcr6*
18. NH-20	Rissen	susceptible	*tetA, sul1, sul3, aadA, gyrB*[a], *parC*[d]
19. NH-26	Rissen	CTX- NAL- STR-TET	*tetA, addA, sul2, gyrB*[b]
20. NH-29	Rissen	susceptible	*tetA, addA, sul3, gyrB*[a], *parC*[d]
21. NH-34	Rissen	TET	*tetA, gyrB*[a]
22. NH-3	Senftenberg	SXT- TET	*tetA, dfrA12, aadA, sul1, sul2, sul3, gyrB*[a], *parC*[e]

No. sample	Serovar	Resistant pattern (MIC and/or agar disc diffusion)	Resistance gene
23. NH-4	Typhimurium	STR- TET	*tetA, tetB, sul2, sul3, aadA, gyrB*[a]
24. NH-17	Typhimurium	STR- TET	*tetA, tetB, aadA, gyrB*[a]
25. NH-23	Typhimurium	TET	*tetA, tetB, aadA, gyrB*[a,b,c]
26. NH-25	Typhimurium	NAL- STR- TET	*tetA, tetB, addA, sul1, sul2, gyrB*[a,b,c]
27. NH-30	Typhimurium	NAL-SXT-TET	*tetA, dfrA12, addA, sul2, sul3, qnrS, gyrB*[b], *parC*[d,e,f]
28. NH-1	Virchow	susceptible	*aadA, gyrB*[a]
29. NH-5	Virchow	susceptible	*tetA, aadA, gyrB*[a]
30. NH-6	Virchow	CTX	*aadA, gyrB*[b]

Legend**:** CTX-cefotaxime, NAL-nalidixic acid, ENR-enrofloxacin, STR-streptomycin, SXT-sulfamethoxazole/trimethoprim, TET-tetracycline
[a]*GyrB* mutation point: G-144 →T, [b]*GyrB* mutation point: C-133 →T, [c]*GyrB* mutation point: G-144 →T and C-133 →T
[d]*ParC* mutation point: C-92 →T; [e]*ParC* mutation point: T-230 →G , [f]*ParC* mutation point: C-92 →T and T-230 →G

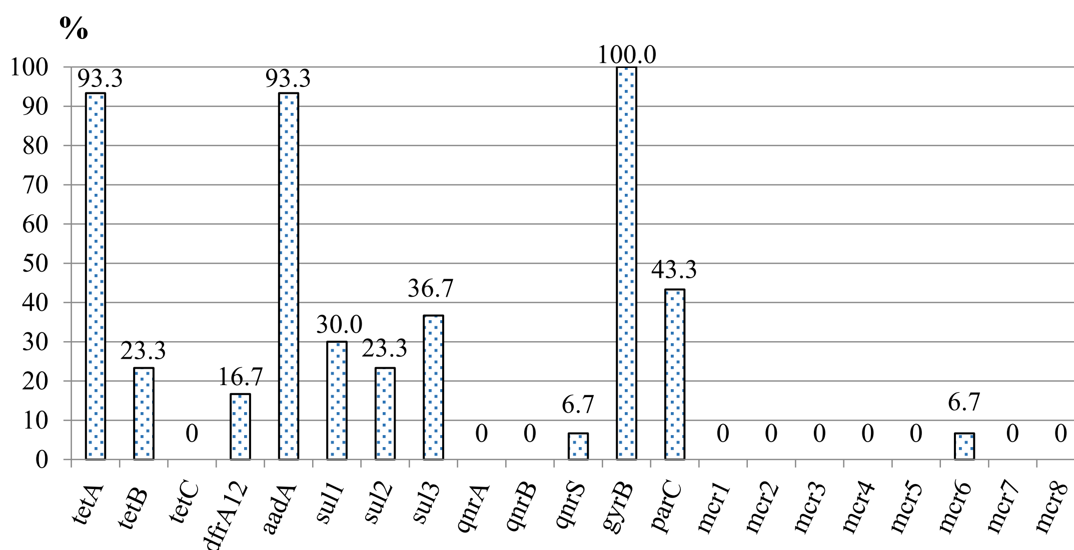

Figure 3 Proportion of the AMR genes found in the 30 Salmonella isolates.

Discussion

Salmonella can be detected at any level along the food preparation chain, including farm, transportation, carcass handling, and food preparation [24]. Environment and equipment, such as the floor, surfaces, knives, cutting boards, and storage places, were possible sources of Salmonella contamination. Therefore, strict hygiene is mandated in food production processes. The main ingredients in Nham are composited ground pork and pig skin derived from many carcasses combined all together and processed by various handlers. According to previous research, the rate of Salmonella in minced pork and pig skin could be as high as 100 % [25]. Homemade Nham recipes use ingredients and create an environment for natural lactic acid-producing bacteria. Fermentation reduces the pH, and hence excludes other pathogens [5]. However, *Salmonella* spp. can grow in a pH range of 5 - 9 [26] but with only limited growth below pH 4.5 [27]. Thai industrial standard 1219 - 2547 (2004) stipulates that a food pH of below 4.6 is safe for consuming fermented products raw. However, the pH of Nham samples in our study ranged from 3.68 - 4.97; this low pH range did not seem to eliminate the Salmonella from the products. In addition to the selection of clean and safe ingredients, many crucial factors during fermentation, such as temperature or delayed growth of lactic acid-producing bacteria, may allow Salmonella to thrive [28]. Unlike homemade products, commercial fermented foods use start culture or standard lactic acid bacteria, such as *Lactobacillus* spp., *Pediococcus* spp., *Streptococcus* spp., and yeast, that grow rapidly and outcompete a number of other pathogens [29].

Salmonella can disseminate into humans via the food chain. In Thailand, *S*. Rissen and *S*. Typhimurium were the most prevalent serovars in pork and ready-to-eat products [30]. Similarly, the top-2 most prevalent Salmonella serotypes discovered from Nham in our study were *S.* Rissen and *S.* Typhimurium. These serotypes were also predominantly found in butcher and pork vendor patients in Lao PDR and Thailand [31]. In addition, *S*. Rissen was frequently detected both in healthy and diarrheal pigs in South Korea [32], and this serovar was mostly encountered in retail pork in Vietnam [33]. In Thailand, *S*. Rissen has been long reported to be one of the most frequent serotypes identified in swine herds [2,34]. In addition, *S.* Rissen has been shown to be efficiently transmitted from swine to humans involved in pork processing [30]. Although *S.* Weltevreden was a common serotype found in human stool, food, and animals in South and Southeast Asia [35], this serotype was not detected in our study.

More than 70 % of Salmonella isolates in our study were resistant to antibiotics. Multidrug-resistant Salmonella was identified at a relatively high rate with 3 to 5 drug class-resistant patterns. Our results indicate that Salmonellae were most resistant to tetracycline, sulfamethoxazole/trimethoprim, and streptomycin. Multidrug-resistant Salmonella has been considered widespread in farm environments [36]. This AMR pattern was in accordance with in-feed and injectable medications commonly used in pig farms in Thailand. This current result was similar to previous studies conducted in Thailand, Vietnam, and South Korea [31-33]. Cephalosporins and quinolones are the first-choice medication for Salmonellosis therapy in human, and colistin is the drug of last resort in treatment [37]. Especially, colistin has been increasingly used as one of the alternative options for treating carbapenem-resistant *Enterobacteriaceae* in humans. Nationwide campaigns dictate the precautious use of these critical agents in livestock; however, this policy seems to be limited to AMR Salmonella of swine origin. From 30 Salmonella isolates, there was only one isolate resistant to enrofloxacin and 5 isolates resistant to cefotaxime. However, the AMR genes correspondent to cephalosporins were not included in our analyses. Enrofloxacin is a quinolone registered for use only in animals. Precautious use of this medicine is strongly recommended since the development of bacterial resistance to this drug might parallel that of other fluoroquinolone drugs. All isolates in our study were susceptible to colistin. This was probably why colistin has not been advised to be used in livestock in Thailand for a few years.

A total of 23 AMR genes were determined in 30 isolates corresponding to colistin, quinolone, streptomycin, tetracycline, sulfamethoxazole, trimethoprim, and colistin resistance. The most prevalently-detected genes were *gyrB* (100 %), *tetA* (93.3 %), and *aadA* (93.3 %). Secondly, sulfonamide resistance genes (*sul1*, *sul2*, *sul3*) were identified. Trimethoprim resistance gene, *dfrA12,* presented as the third-most frequent. Whereas the quinolone-resistant *qnrS* and the colistin-resistant *mcr*6 genes were the least detected, only quinolone resistance in the determinant region of Enterobacteriaceae is associated with a point mutation in *gyrA, gyrB* and *parC* [37]. However, quinolone resistance in Salmonella can be mediated by other genes associated to its plasmid that were not evaluated in this study. Plasmid-mediated colistin resistance conferred by the *mcr*1, *mcr*2, *mcr*3, *mcr*4, and *mcr*5 genes have already been identified in several serovars of *Salmonella enterica* [38]. We did not find a colistin-resistant phenotype but detected 2 isolates harboring the *mcr6* gene. Generally, the prevalence of colistin resistance in Salmonella isolated from healthy animals is low [39]. In addition, there were 7 Salmonella isolates carrying AMR genes but susceptible to the correspondent antibiotics. The discrepancy between genotype and phenotype can be explained by a lack of gene expression, ancestral genes that require mutation, or other genes required in parallel to confer resistance traits. Otherwise, bacteria may resist medication by physical or chemical adaptation without any change in their genotypes.

Conclusions

There is an urgent need for Hazard Analysis Critical Control Point (HACCP) in meat processing and homemade Nham production processes due to a relatively high Salmonella prevalence and antibiotic resistance found in the product. Such steps to mitigate Salmonella infections include selecting Salmonella-free pork, finding an appropriate storage place and temperature, applying good hygiene, using standard fermentation cultures, and evaluating product safety. Another alternative method for pathogen elimination in pork is radiating, freezing and then thawing the meat before producing Nham. An investigation into the food supply chain contaminated with MDR Salmonella and the interplay between animal hosts, food, and environment should be undertaken. Governmental strengthening of the surveillance system to control and prevent the dissemination of MDR Salmonella between animals or animal products to humans is indicated by our data.

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