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RESEARCH ARTICLE

Occurrence and Antimicrobial Resistance in *Salmonella enterica* from Sport Animals and Livestock in Southern Thailand

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A B S T R A C T

Sport animals and livestock farms are sources of pathogens, including *Salmonella* spp. that were resistant to antimicrobial agents and carries antibiotic resistance genes. This study aimed to isolate *Salmonella* spp. from sports animals and livestock farms in Southern Thailand, and to characterize the antimicrobial resistance profile of the isolates. A total of 241 samples were collected from sport animals (fighting cocks, fighting bulls, and riding horses) and swine breeding farms. The suspected *Salmonella* colonies were identified using Matrix-Assisted Laser Desorption/ Ionization Time-Of-Flight (MALDI-TOF) MS. Antimicrobial susceptibility against the isolates and detection of the antibiotic resistance genes were investigated. It was found that 20.3% (49/241) of the animal specimens were positive for *Salmonella* spp. The swine samples showed the highest prevalence of *Salmonella* spp., with a prevalence of 31.5% and 62.6% in feces and soil of the farms, respectively. A total of 98 *Salmonella* spp. isolates were isolated and tested for their antimicrobial susceptibility. Tetracycline resistance was the most common (48.1%), followed by ampicillin (40.4%). The minimum inhibitory concentration (MIC) of tetracycline ranged from ≤1 to ≥16 µg/mL across all *Salmonella* isolates. Highly resistant strains exhibited MIC_{90} values of ≥16 µg/mL, indicating that 90% of the isolates were inhibited at this concentration. Among the tetracycline resistance genes, *tet*A and *tet*G genes were the most prevalent, detected in 85.0% of the samples, followed by *tet*B (47.5%). For species identification, 7 isolates that showed multi-drug resistance (MDR) were closely similar to *S. enterica* as detected by *inv*A gene sequencing. These findings contribute to understanding and controlling the spread of antibiotic resistance genes in *Salmonella* spp. from animals.

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INTRODUCTION

Sports animals such as fighting cocks, fighting bulls, and riding horses are valuable companions and competitors that have long been linked with a culture in Southeast Asia, including Thailand (Hata *et al*., 2021). Furthermore, sports animals are also livestock animals that have a significant economy. Antibiotics have proven to be highly advantageous for human health, animal welfare, and the production of food. The widespread use of antibiotics has largely contributed to the evolution of antimicrobial resistance (AMR), which is a major public health concern worldwide (Holmes *et al.*, 2016). Furthermore, AMR has emerged as one of the most important and immediate dangers to public health, seriously impeding the effective prevention and treatment of chronic diseases (GBD, 2021). Importantly, ARM can cause death and disability in humans and animals, which has a huge impact on the

economy and health (Dadgostar, 2019). Several species of bacteria have been reported to be resistant to antibiotics, as well as multidrug-resistant bacteria carrying antibiotic-resistant genes, such as *Staphylococcus aureus*, *Escherichia coli* as well as *Salmonella* spp. (Mengistu *et al*., 2022). Importantly, these antibiotic-resistant pathogens can carry antibiotic resistant genes, and serve as zoonotic pathogens. Therefore, the emergence of antibiotic-resistant *Salmonella* spp. becomes an important public health concern worldwide (Jiang *et al*., 2019).

Salmonella species are considered as one of the major causes of foodborne diseases worldwide. *Salmonella* is a Gram-negative bacterium found in the intestinal tract in various species of animals such as chickens, cows, horses, and pigs. This bacterium, which is extremely widespread, comprises over 2600 distinct serovars that are classified into typhoidal and non-typhoidal *Salmonella* serovars (Gal-Mor *et al*., 2014). Some serotypes of *Salmonella* such as *S.* Typhimurium are important pathogens that cause inflammation and necrosis of the small and large intestines, as well as diarrhea in animals (Meurens *et al*., 2009). Moreover, there are numerous serotypes of *Salmonella* spp. that can sequentially and transiently infect individuals and result in food poisoning. Additionally, the bacteria are a zoonotic human pathogen linked to foodborne illnesses in humans related to animals as well as the environments on farms (Fakruddin *et al*., 2017).

Nakhon Si Thammarat Province, a large city in Southern Thailand, is located in a tropical climate zone. The province is one of the leading areas of production of many sport animals such as fighting cocks, fighting bulls, and riding horses in Thailand. In addition, this city is a production area for swine, which is an important livestock animal in Thailand. Animal feces have been used as natural fertilizer in plant agriculture, indicating the source of antibiotic resistant pathogens that may carry antibiotic resistant genes. Therefore, this study aimed to investigate prevalence and characterization of *Salmonella* spp. from the sport animals and livestock farms in Southern Thailand. Antimicrobial susceptibility of *Salmonella* spp. and detection of antibiotic resistance genes presented in the isolates were carried out.

MATERIALS AND METHODS

Ethics approval: The present study was approved by the Institutional Animal Care and Use Committee of Walailak University (Ref. No. WU-AICUC-63-023). All the experiments were performed under the regulation of biosafety for scientific experiments (Ref. No. WU-IBC-66-018) of the Walailak University, Nakhon Si Thammarat, Thailand.

Study period and location: The present study was conducted from August 2023 to November 2023. Samples of animal feces and soil in the farms were collected from sport animals and livestock farms, located in Nakhon Si Thammarat Province, Southern, Thailand. All experiments were carried out at the bacterial laboratory, Walailak University, Nakhon Si Thammarat, Thailand.

Sample collection and preparation: A total of 241 samples were collected from 72 farms the sports animals including fighting cocks (10 farms), fighting bulls (21 farms), and riding horses (6 farms), and livestock animal farms (swine; 35 farms) in Nakhon Si Thammarat province, Thailand. Samples included fecal samples on farms and environmental ones including soil samples. It was noticed that the collection of sports animals including cocks, bulls, and riding horses was conducted according to the approved ethics. Fighting cock feces were collected using a cotton swab inserted into the chicken's cloaca and placed in a sterile container. While, fresh fecal pats of fighting bull and riding horse were collected. However, at the swine farm collection was done from the feces at a pile behind the stall without any contact with the swine. Fresh feces were also obtained from a fecal pile of the animal, including 43 bulls, 39 cocks, 33 riding horses and 54 swine. In addition, 72 soil samples were collected from the animal pen area using a sterile scoop and placed in a sterile container. All samples were kept in sterile containers, preserved between 2-8°C and transported to the laboratory within 8h of collection.

Isolation and identification of *Salmonella* spp: All samples underwent isolation of *Salmonella* spp. according to International Organization for Standardization (ISO) 6579 (ISO 2017) (Jiang *et al*., 2019). Samples were added to buffered peptone water (BPW) (Oxoid, UK) at a ratio of 1:10 and incubated at 37 °C for 18-24 h. Subsequently, 100 µL of pre-enriched culture was inoculated into modified semisolid Rappaport-Vassiliadis medium (MSRV) (Oxoid, UK) and incubated for 18-24h, at 41.5°C to select *Salmonella* and to inhibit other bacteria. The bacteria on MSRV agar were streaked on Xylose Lysine Deoxycholate agar (XLD) (Oxoid, UK), incubated at 37°C for 18-24h. Suspected colonies of *Salmonella* spp. (black center and slightly transparent reddish zone) were streaked on Tryptic Soy Agar (TSA) (Oxoid, UK) and incubated at 37°C for 18-24 h. The suspected *Salmonella* colonies were identified using MALDI-TOF MS (MALDI biotype, Bruker, Bruker Daltonik GmbH, Germany).

Antimicrobial Susceptibility Testing: The antimicrobial susceptibility test of the 98 *Salmonella* isolates was performed by the VITEK® 2 AST-GN96 test kit cards, and the VITEK® 2 COMPACT machine (bioMérieux, Marcy l'Etoile, France). The assay was performed to determine the minimum inhibitory concentration (MIC) based on microdilution method. The 17 antimicrobial agents tested were amoxicillin/clavulanic acid (AMC), ampicillin (AM), cefalexin (CN), cefalotin (CF), cefoperazone (CFP), cefquinome (CEQ), ceftiofur (CFT), enrofloxacin (ENR), florfenicol (FFC), flumequine (UB), gentamicin (GM), imipenem (IPM), marbofloxacin (MRB), neomycin (N), tetracycline (TE), ticarcillin/ clavulanic acid (TCC), trimethoprim/sulfamethoxazole (SXT). The used antibiotics were recommended against the Gram-negative bacteria including *Salmonella* spp. The results were presented as antimicrobial susceptibility (sensitive, intermediate, and resistant) based on the MIC and the MIC values. The interpretation of categories of susceptible, intermediate or resistant was conducted by an advanced Expert System™ based on the global Clinical and Laboratory Standards Institute guidelines and natural resistance guidelines (Clinical and Laboratory Standard

Institute 2019). The results were presented as percent resistance that calculated by the numbers of isolates that resistant to each antibiotic per the total of the bacteria. In addition, the MIC, MIC_{50}, and MIC_{90} values of each antibiotic against the isolates were recorded. MIC_{50} and MIC_{90} values represent the MIC values at which ≥50% and ≥90% of the isolates in a test population are inhibited, respectively. Multidrug resistance (MDR) refers to the ability of bacteria to resist antibiotics from more than three different classes.

Detection of antibiotic resistance genes: Forty *Salmonella* spp. isolates that showed phenotypic resistance to antimicrobial agents were selected to detect the possession of relevant resistance genes by Polymerase chain reaction (PCR) assay. Genomic DNA was extracted from an overnight bacterial culture with the Presto™ Mini gDNA Bacteria Kit (Geneaid, New Taipei City, Taiwan) according to the manufacturer's instructions. The antimicrobial resistance (AMR) genes selected for detection and the corresponding published detection assays used were: *bla*$_{TEM}$ (Randall *et al*., 2004) for Ampicillin, *aph*A1 (Jaja *et al*., 2019) for Neomycin, *qnr*A (Wang *et al*., 2021) for Enrofloxacin, *oqx*A (Wang *et al*., 2021) for Flumequine, *flo*R (EI-Tayeb *et al*., 2017) for Florfenicol, *tet*A (EI-Tayeb *et al*., 2017), *tet*B (EI-Tayeb *et al*., 2017), *tet*G (Zhu *et al*., 2017) for Tetracycline, and *dfr*A (Chuanchuen & Padungtod, 2009), *Sul*II (Ei-Sharkawy *et al*., 2017) for Trimethoprim/Sulfonamides.

The PCR mixture comprised of 12.5 µL 2x Master Mix with buffer (Bio-Helix, Taiwan), 0.5 µL of DNA template, 0.25 µM each of the forward and reverse primer and nuclease-free water to make a final volume of 25 µL. All PCR assays were carried out using a GeneAmp PCR System 9700 (Thermo Fisher Scientific, MA, USA). Negative controls (nuclease-free water) were used in all reactions. Amplified genes were analyzed by 1.5% agarose gel electrophoresis (Vivantis, Shah Alam, Malaysia) and visualized by ultraviolet trans-illuminator.

DNA sequencing and bioinformatic analysis: The isolates of *Salmonella* spp. that showed multi-drug resistance were selected for further identification. The genomic DNA was extracted as described above. The PCR was performed using primers for species confirmation. A set of primer that target the invA virulence gene was used to detected Salmonella species (invA-F: 5′- GCTCTTTCGTCTGGCATTATC-3′ and invA-R: 5′-GCATCAAATCAAAATAGACCG-3′) (Okidi et al., 2022). A 25 µL of PCR mixture was performed in a thermocycler (Bio-Rad T100 Thermal Cycler, Bio-Rad, USA) and the PCR condition included: 1 cycle of initial denaturation (95ºC, 5 min), followed by 35 cycles of denaturation (95ºC, 35 s), annealing (50ºC, 35 s), and extension (72ºC, 45 s), and last one step of extension (72ºC, 7 min). Then, PCR product was run in 1.2% agarose gel under 0.5X TAE buffer (130 volts, 30 min). The sample that presents DNA band at approximately 1,000 bp was suspected as Salmonella spp. Salmonella suspected band was further sequenced using Sanger sequencing. The obtained DNA sequence was analyzed using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). In addition, the sequences were subjected to establish a phylogenetic tree using MEGA-X software (https://www.megasoftware.net) under neighbor-joining method with 1,000 bootstrap replications.

Statistical analysis: The statistical analysis was carried out using R-programming language version 4.0.2. Descriptive statistics were used to describe the data. The Chi-square and Fisher's exact test were used to determine the relationship between the prevalence of *Salmonella* spp. and types of animals and samples. All statistical analyses were performed under a 95% confidence interval, and $P<0.05$ was considered a significant difference.

RESULTS

Detection of *Salmonella* spp: A total of 241 samples were collected from the animal farms. The results showed that 20.3% (49/241 samples) were positive of *Salmonella* detection that found 98 *Salmonella* isolates. The bacteria were gram-negative, non-endospore-forming, and hydrogen sulfide producing bacteria. It was observed that samples from swine including feces and the soil showed the highest prevalence of *Salmonella* spp., compared with other animal species (Table 1). Amount this, 17 samples (31.5%) and 22 samples (62.6%) were detected from the swine feces and the soil in the farms, respectively. It was noticed that the amount of the samples collected from the soil was statistically significant, compared with the feces ($P<0.05$). In addition, the samples of riding horse and bull feces were *Salmonella* positive as 5 samples (15.2%) and 4 samples (9.3%), respectively. However, *Salmonella* spp. from the samples of cock feces was not detected in this study.

Antimicrobial susceptibility: Antimicrobial susceptibility profiles of the isolates of *Salmonella* spp. were investigated using several classes of antibiotics according to CLSI by microdilution assay. As shown in Table 3, both the isolates from the feces and the soil were resistant to tetracycline with the percent resistance of 48.1% and 32.6%, respectively. Ampicillin was the second resistant antibiotic detected in the isolates. It was highlighted that 8 isolates showed multi-drug resistance against at least 3 antibiotics (Table 3). On the other hand, the isolates were susceptible to cephalosporin groups, aminoglycosides, and beta-lactams except for ampicillin. However, antibiotic resistance against some antibiotics such as flumequine, florfenicol, trimethoprim/ sulfamethoxazole, and enrofloxacin has also been detected.

The MIC values of the isolates of *Salmonella* were presented in Table 2. The results revealed that the MIC values of antibiotics against *Salmonella* spp. were related to those of the antibiotic susceptibility profiles. The MIC of tetracycline ranged from ≤1 to ≥16 µg/mL across all *Salmonella* isolates. Highly resistant strains exhibited MIC_{90} values of ≥16 µg/mL, indicating that 90% of the isolates were inhibited at this concentration (Table 2). In addition, the MIC_{50} and MIC_{90} of ampicillin against *Salmonella* isolates were ≤2 and ≥32 µg/mL, respectively. The MIC values of other antibiotics against the isolates were presented in Table 2.

Table 1: Prevalence of *Salmonella* spp. in feces and soil samples collected from four types of animal farms

Animal farms	Feces		Soil		P-value
	Numbers of samples	Positive samples	Numbers of samples	Positive samples	
		No. samples (%)		No. samples (%)	
Fighting bulls	43	4 (9.3%)	21	0 (0%)	0.290
Fighting cocks	39	0 (0%)	10	1 (10.0%)	0.200
Riding horses	33	5 (15.2%)	6	0 (0%)	0.570
Swine	54	17 (31.5%)	35	22 (62.6%)	0.007

Table 2: Antimicrobial resistance and minimal inhibitory concentration of antibiotics against of *Salmonella* spp. isolates (n=98)

Antibiotic	Feces (n=52)			Soil (n=46)		
	R (%)	MIC range	MIC_{90}	R (%)	MIC range	MIC_{90}
Ampicillin	40.4	≤ 2 - ≥ 32	≥ 32	19.6	≤ 2 - ≥ 32	≥ 32
Amoxicillin/Clavulanic acid	0.0	≤ 2 - 8	8	0.0	≤ 2 - 8	4
Ticarcillin/Clavulanic acid	0.0	≤ 8 - 64	16	0.0	≤ 8 - 16	16
Cefalexin	0.0	≤ 4 - 16	16	0.0	≤ 4 - 8	≤ 4
Cefalotin	0.0	≤ 2 - 8	8	0.0	≤ 2 - 4	8
Cefoperazone	0.0	≤ 2- 8	≤ 4	0.0	≤ 1- ≤ 4	≤ 4
Ceftiofur	0.0	≤ 1 - 4	≤ 1	0.0	≤ 1 - 2	≤ 1
Cefquinome	0.0	≤ 0.5 - 1	≤ 0.5	0.0	≤ 0.5 - 2	≤ 0.5
Imipenem	0.0	≤ 0.25 - ≤ 0.5	≤ 0.25	0.0	≤ 0.25 - ≤ 0.5	≤ 0.25
Gentamicin	0.0	≤ 1- ≤ 1	≤ 1	0.0	≤ 1- ≤ 1	≤ 1
Neomycin	1.9	≤ 2 - ≥ 64	≤ 2	4.3	≤ 2 - ≥ 64	≤ 2
Flumequine	15.4	≤ 1 - ≥ 32	≥ 16	6.5	≤ 1 - ≥ 32	≤ 1
Enrofloxacin	3.8	≤ 0.12 - ≥ 4	1	0.0	≤ 0.12 - 1	≤ 0.12
Marbofloxacin	0.0	≤ 0.5 - 2	1	0.0	≤ 0.5 - 1	≤ 0.5
Tetracycline	48.1	≤ 1 - ≥ 16	≥ 16	32.6	≤ 1 - ≥ 16	≥ 16
Florfenicol	5.8	4 - ≥ 32	8	6.5	4 - 16	8
Trimethoprim/Sulfamethoxazole	5.8	≤ 20 - ≥ 320	≤ 20	0.0	≤ 20 - ≤ 20	≤ 20

Table 3: Phenotypic and genotypic antimicrobial resistance profile of *Salmonella* spp. isolates (n=40)

Patterns of resistance	Numbers of isolates (%)			Genotypic antimicrobial resistance (No.)	
	Feces (n=23)	Soil (n=17)	Total (n=40)	Feces (n=23)	Soil (n=17)
FFC	0	2 (11.8)	2 (5.0)	-	*flo*R (2)
TE	0	3 (17.6)	3 (7.5)	-	*tet*A (3), *tet*B (3), *tet*G (3)
AM-TE	16 (69.6)	6 (35.3)	22 (55.0)	bla_{TEM} (16), *tet*A (15), *tet*B (10), *tet*G (14)	bla_{TEM} (5), *tet*A (4), *tet*B (3), *tet*G (6)
UB-TE	2 (8.7)	3 (17.6)	5 (12.5)	*opx*A (0), *tet*A (2), *tet*B (0), *tet*G (2)	*opx*A (0), *tet*A (3), *tet*B (0), *tet*G (3)
AM-TE-FFC	0	1 (5.9)	1 (2.5)	-	bla_{TEM} (1), *tet*A (0), *tet*B (1), *tet*G (0), *flo*R (1)
AM-N-TE	0	2 (11.8)	2 (5.0)	-	bla_{TEM} (2), *aph*A1(2), *tet*A (2), *tet*B (2), *tet*G (2)
AM-UB-TE-FFC	1 (4.3)	0	1 (2.5)	bla_{TEM} (1), *opx*A (0), *tet*A (1), *tet*B (0), *tet*G (1), *flo*R (1)	-
AM-TE-FFC-SXT	1 (4.3)	0	1(2.5)	bla_{TEM} (1), *tet*A (1), *tet*B (0), *tet*G (1), *flo*R (1), *dfr*A(0), *Sul* 2 (0)	-
AM-UB/ENR-TE-SXT	2 (8.7)	0	2 (5.0)	bla_{TEM} (2), *opx*A (0), *qnr*A (0), *tet*A (2), *tet*B (0), *tet*G (2), *dfr*A(0), *Sul*2-(0)	
AM-N-UB-TE-FFC	1 (4.3)	0	1 (2.5)	bla_{TEM} (1), *aph*A1 (1), *opx*A (0), *tet*A (1), *tet*B (0), *tet*G (0), *flo*R (1)	-

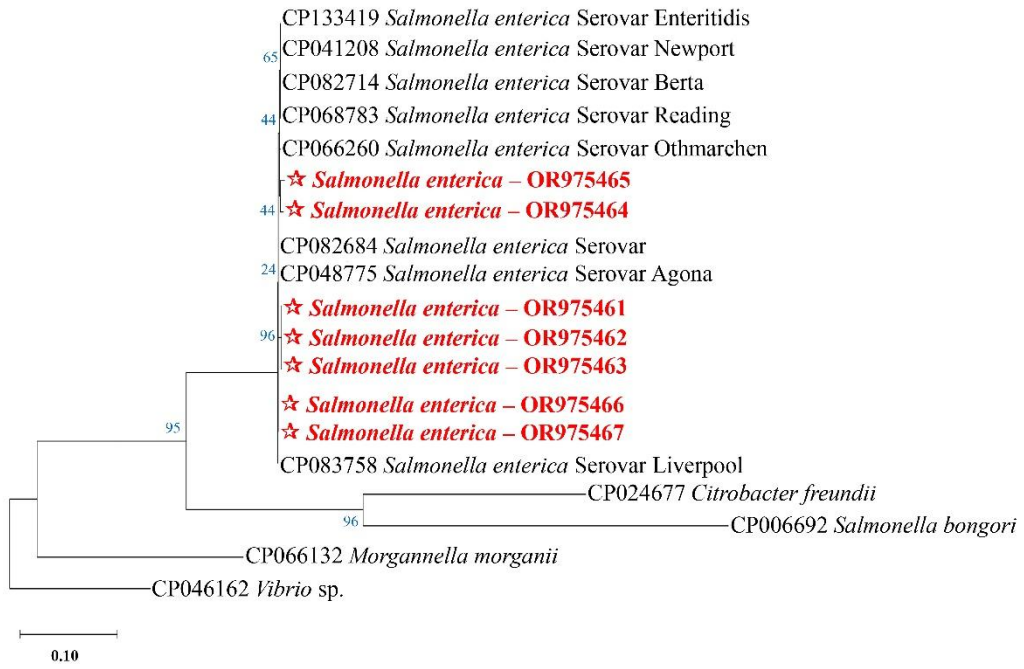

Fig. 1: Phylogenetic tree of *invA* gene of MDR *Salmonella enterica*. *Morganella morganii* and *Vibrio* sp. are provided as an outgroup; and stars indicate positive samples from this study.

Antibiotic resistance genes: To confirm the phenotypic antimicrobial resistance shown molecular resistance genes were detected on the same isolates. The prevalence of tetracycline resistance genes was the main resistance genes detected in the *Salmonella* isolates that was related to the results of the antimicrobial susceptibility (Table 3). Among the tetracycline resistance genes, *tet*A and *tet*G genes were the most prevalent, detected in 85.0% of the sample, followed by *tet*B (47.5%). It was found that 72.5% of detection of the bla_{TEM} gene associated with ampicillin resistance was observed in the isolates. For the ten identified resistance genes, the isolates had the highest positive proportion, except for *qnr*A, *oqx*A, *dfr*A1, and *sul*2 that presented antibiotic resistance against the drugs.

Bioinformatic analysis: The obtained DNA sequence was performed sequence alignment using BLAST in GenBank. All amplicons of the selected MDR *Salmonella* spp. were closely similar to *S. enterica* (99.2-100.0% of query cover and 99.7-100.0% of identity). The phylogenetic tree of partial sequences of *inv*A indicated that the sequences of MDR *Salmonella* spp. in this study were grouped in the same linage of *S. enterica* (Fig. 1). Moreover, the partial sequences of *inv*A gene were prominently separated from the closely species, *S. bongori*. All sequenced *S. enterica* amplicons were submitted to GenBank with accession no. OR975461- OR975467.

DISCUSSION

Salmonella causes a public health risk by generating widespread clinical foodborne illnesses and extensive antimicrobial resistance (Parada *et al*., 2022). The present study revealed the prevalence of *Salmonella* spp., the antibiotic resistance as well as the genes related to the antibiotic resistance in both sports animals and livestock animals. The results revealed that the samples from swine including feces and the soil showed the highest prevalence of *Salmonella* spp., compared with other animal species. It was noticed that the amount of the isolates collected from the soil was statically significant, compared with the feces. Isolation of *Salmonella* spp. from the swine feces in Thailand has been reported (Anuchatkitcharoen *et al*., 2020). Moreover, the *Salmonella* detection from swine, carcasses and workers in slaughterhouses, retail pork and butchers in fresh markets has been documented (Sinwat *et al*., 2016). Therefore, the detection of *Salmonella* spp. in pork, workers in slaughterhouses, and the farm may be considered as one health concern. The most common serotypes of *Salmonella* spp. isolated from swine husbandry in Thailand were Typhimurium and Rissen (Sinwat *et al*., 2016). Unfortunately, the serotypes of the isolated *Salmonella* spp. did not determine in this study. It is accepted that *inv*A gene, an invasion protein A gene, has been used to identify and confirm *Salmonella* spp. because the *inv*A gene is only found in *Salmonella* spp. The phylogenetic tree of partial sequences of *inv*A indicated that the sequences of MDR *Salmonella* spp. in this study were grouped in the same linage of *S. enterica*.

The isolation of *Salmonella* spp. from the sport animals including fighting bulls and riding horse was also presented in this study; however, the prevalence was less than in swine. Our results were similar to those studies on the prevalence of this pathogen in other parts of Thailand (Padungtod & Kaneene, 2006). In addition, the prevalence of *Salmonella* spp. in both fighting bulls (Padungtod and Kaneene, 2006) and riding horses (Goni *et al*., 2023) has been documented. Due to fighting by the sport, the animals may increase the transmission of the pathogen from *Salmonella* infected animals to non-*Salmonella* infected animals. It has been known for a long time that the feces of both fighting bulls and riding horses have been used as natural fertilizer plant agriculture. Hence, the contamination of *Salmonella* spp. with the feces may increase the pathogen in ready-to- eat vegetables due to the food chain processes. The detection of several serotypes of *Salmonella* spp. from 80 vegetable samples has been reported (Meunsene *et al*., 2021). However, the contamination of this pathogen in the vegetable may occur from several factors including sanitation practices and hygiene during growth, harvest, storage, and distribution (Meunsene *et al*., 2021).

In this study, tetracycline was the most predominant resistance antibiotic found in *Salmonella* isolates from both sport animals and livestock animals. Relatively, *tet*A and *tet*G genes were found to be the most predominant resistance gene that related to the resistance to tetracycline. Furthermore, the detection of tetracycline resistance and the predominant *tet*A gene were also observed in the *Salmonella* spp. isolated from the soils in the farms, indicating the transmission of the gene carried-pathogen to the environments. The results were similar to Wongtawan and colleagues that reported the most predominant *tet*A detected *E. coli* isolates from sports animals (Wongtawan *et al*., 2022). Tetracycline is used to treat the infections caused by both Gram-negative and Gram-positive pathogens in animals as well as human (Græsbøll *et al*., 2017). Tetracycline resistance is primarily associated with chromosomal mutations, modifications to ribosomal binding sites, and mobile genetic elements. Key resistance strategies include efflux pumps, ribosomal protection proteins, and enzymatic inactivation of tetracycline drugs. Among these, the most prevalent determinants are the *tet*A, *tet*B, *tet*C, *tet*D, and *tet*G genes, which encode efflux pumps that actively expel tetracyclines from the cells (Pavelquesi *et al*., 2021). In livestock farming, tetracyclines have been extensively used for decades to treat bacterial infections (Mala *et al*., 2021). Importantly, these antibiotic-resistance genes can be horizontally transferred to other pathogens including *Salmonella* spp (Oladeinde *et al*., 2019). It was observed that ampicillin was the second resistant antibiotic found in the isolates. Moreover, a high prevalence of bla_{TEM}, a resistance gene that is related to ampicillin resistance, was presented in the isolates. It is well-known that ampicillin is used to treat certain infections that are caused by bacteria. However, the high resistance to ampicillin has been detected in many bacteria via several mechanisms of actions such as beta-lactamase production, efflux pump system, as the presence of *bla* cluster genes including bla_{TEM} genes (Gargano *et al*., 2022). In addition, extended spectrum beta-lactamase genes that occurred in the high resistance to the drug in beta-lactam groups have been reported in *Salmonella* spp. (Di Marcantonio *et al*., 2022). It has been reported that mobile genomic elements promote the migration and dissemination of antimicrobial resistance genes, resulting

in the AMR accumulation, interaction, and persistence (Jia *et al*., 2023).

Conclusions: The present study investigated the prevalence of *Salmonella* spp. in sport animals and livestock farms in Southern Thailand. *Salmonella* spp. isolates were most frequently identified in swine-related samples (feces and soil) compared to other animal species. Tetracycline and ampicillin emerged as the predominant antibiotics associated with resistance, with a subset of isolates exhibiting MDR to at least three antibiotic groups. Genetic analysis revealed that all selected MDR isolates were closely related to *S. enterica*, with *tet*A and *tet*G being the most prevalent tetracycline resistance genes, followed by *tet*B. These findings contribute to understanding and controlling the spread of antimicrobial resistance genes in *Salmonella* spp. from animals.

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Authors contribution: Conception and design of the study: RN, TW, RB, PS, and WM. Acquisition of data: RN, RB, PS, TW, and WM. Analysis and/or interpretation of data: RN, RB, PS, and WM. Drafting the manuscript: RN, RB, PS, MLP, VN, and WM. Critical review/ revision: VN, MLP, PP, and WM. Contribution of reagents, materials, analysis tools or data: VN, AP, PP, and MLP.

Conflict of interest: The authors declare no conflict of interest.

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