

Whole-genome characterisation of multidrug resistant monophasic variants of *Salmonella* Typhimurium from pig production in Thailand

Prapas Patchanee¹, Prawitchaya Tanamai¹, Phacharaporn Tadee², Matthew D. Hitchings³, Jessica K. Calland⁴, Samuel K. Sheppard^{4,5}, Dethaloun Meunsene⁶, Ben Pascoe^{4,5} and Pakpoom Tadee¹

¹Integrative Research Center for Veterinary Preventive Medicine, Department of Food and Animal Clinics, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand

²Faculty of Animal Science and Technology, Maejo University, Chiang Mai, Thailand

³Swansea University Medical School, Swansea University, Swansea, UK

⁴The Milner Centre for Evolution, Department of Biology and Biochemistry, University of Bath, Bath, UK

⁵Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand

⁶Department of Veterinary Medicine, Faculty of Agriculture, National University of Laos, Vientiane, Laos PDR

ABSTRACT

Background: Monophasic *Salmonella* Typhimurium or *S. enterica* 1,4,[5],12:i:- is among the top five serotypes reported in Thailand. In this study, nineteen monophasic *S. Typhimurium* from the pig production chain in Chiang Mai and Lamphun provinces during 2011–2014 were sequenced and compared to a globally disseminated clone. Isolates were probed in silico for the presence of antimicrobial resistance genes and *Salmonella* virulence factors, including Pathogenicity Islands.

Results: All isolates were from sequence type 34 (ST-34) and clustered similarly in core and pangenome genealogies. The two closest related isolates showed differences in only eighteen loci from whole-genome multilocus sequence typing analysis.

All 19 isolates carried aminoglycoside and beta-lactam class resistance genes and genes for five or more different antibiotic classes. Seven out of 14 known SPIs were detected, including SPI-5, SPI-13 and SPI-14, which were detected in all isolates.

Conclusions: The multi-drug resistant clone, ST-34 was sampled at all stages of pork production. This clone has infiltrated global agricultural processes and poses a significant public health risk. Differences in the core and accessory genomes of the isolates we collected suggest that strains persist though the pork production process, with evidence of mutation within the core-genome and horizontal acquisition of genes, potentially via sharing of pathogenicity islands and plasmids. This highlights the importance of surveillance and targeted intervention measures to successfully control *Salmonella* contamination.

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Corresponding authors

Ben Pascoe, B.Pascoe@bath.ac.uk

Pakpoom Tadee,

pakpoom.t@cmu.ac.th

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INTRODUCTION

Salmonella is a globally important cause of diarrhoeal diseases (*World Health Organization (WHO), 2015*). Annually, there are ninety-million cases with 150,000 deaths of food-borne salmonellosis estimated worldwide (*Majowicz et al., 2010*). Disease in humans is associated with contaminated or undercooked meats (*Bangtrakulnonth et al., 2004; Mossong et al., 2007; Campioni, Moratto Bergamini & Falcao, 2012*). Symptoms observed include acute fever, nausea, vomiting and diarrhoea within 8–48 h of infection (*Jawetz, Melnick & Adelberg, 1981*). Prolonged sickness leads to a larger expenditure on health care and an increased risk of death (*Tanwar et al., 2014*).

Salmonella can be classified into serotypes using a serum agglutination test (*Popoff & Le Minor, 1992*) and to date more than 2,500 serotypes have been defined (*Grimont & Weill, 2007*). Monophasic *S. Typhimurium* variants (1,4,[5],12:i:-) that lack phase two flagellar antigens have become prevalent worldwide and present a significant public health risk through contamination of the food production chain (*Gymoese et al., 2017; Petrovska et al., 2016; Yang et al., 2015*). *Salmonella* 1,4,[5],12:i:-isolates represent emergent clones that are often multi-drug resistant (*Skarżyńska et al., 2017; Yang et al., 2015*) and, along with other monophasic *S. Typhimurium*, are among the main serotypes found in pig production in northern Thailand (*Tadee et al., 2015*). Evidence suggests that contaminating strains persist from the farm, through the slaughterhouse, to marketplace (*Patchanee et al., 2016*). The expansion of the monophasic *S. Typhimurium* clade (1,4,[5],12:i:-) is associated with the acquisition of the *Salmonella* genomic island four (SGI-4; up to 87 genes), which contains genes associated with resistance to copper, silver, mercury or arsenic and is thought to have enabled the proliferation of antimicrobial resistance determinants within the pork production industry (*Branchu et al., 2019; Patchanee et al., 2016; Tadee et al., 2015*). Genomic islands are large, flexible genome elements that carry sets of genes that can confer novel phenotypes, such as heavy metal resistance in this case (*Hallstrom & McCormick, 2015*). Furthermore, *Salmonella* pathogenicity islands (SPIs), a subset of the genomic islands, can also play a pivotal role in virulence and severity (*Banaszkiewicz et al., 2019; Elder et al., 2018; Marcus et al., 2000*), often associated with specific host niches (*Amavisit et al., 2003; Foley et al., 2013*). Together, emerging multidrug resistance and enhanced virulence, make successful treatment of salmonellosis patients increasingly difficult (*Tanwar et al., 2014*).

Global surveillance efforts and development of large genome databases for comparative genomics have enabled quantification of variation within human infection and agricultural isolates (*Jolley, Bray & Maiden, 2018; Ribot & Hise, 2016*). The improved discriminatory power of next generation sequencing, with increased throughput, scalability and speed has led to improvements in surveillance and source attribution (*Henri et al., 2017; Kwong et al., 2015; Toro et al., 2016*). In silico identification and annotation of putative genes, and a better understanding of sequence variation through multilocus sequence typing (MLST) schema have informed epidemiological studies (*Bradley et al., 2015; Zankari et al., 2013; Henri et al., 2017; Sheppard, Jolley & Maiden, 2012; Toro et al., 2016*).

Table 1 Origin and characterisation of monophasic *Salmonella* Typhimurium isolated from different sources in Chiang Mai and Lamphun.

Strains ID	Location ^a	Source	Sampling Date	ABO-resistance pattern ^b
A541006	Farm 1	Pig's faeces	2 November 2011	AMP,S,CTX,TE
A541007	Farm 1	Pig's faeces	2 November 2011	AMP,S,CTX,TE
A541011	Farm 1	Pig's faeces	15 November 2011	AMP,C,CTX,TE
A541012	Farm 1	Pig's faeces	15 November 2011	AMP,S,NA,CTX,TE
A541013	Farm 1	Pig's faeces	15 November 2011	AMP,S,TE
A541024	Farm 2	Pig's faeces	20 December 2011	AMP,S,CTX,TE
A541025	Farm 2	Pig's faeces	20 December 2011	AMP,C,S,CTX,TE
A543008	Farm 3	Stable floor swab	2 June 2012	AMP,S,TE
A543009	Farm 1	Stable floor swab	27 June 2012	AMP,C,S,CTX,TE
A543010	Farm 2	Stable floor swab	3 July 2012	AMP,SXT,C,S,CTX,TE
21-JRSP3	SLH A	Carcass splitter swab	5 May 2013	AMP,S,TE
172-BT E22	SLH B	Carcass swab	2 June 2013	AMP,S,TE
173-BT E24	SLH B	Mesenteric lymphnode	2 June 2013	AMP,S,TE
117-LP L4	SLH C	Lairage area floor swab	9 June 2013	AMP,C,S,CTX,TE
60-JRD3	SLH A	Knife swab	4 August 2013	AMP,S,TE
193-BT D11	SLH B	Carcass swab	2 September 2013	AMP,S,TE
TP3	Market X	Butchering pork product	29 June 2014	AMP,C,TE
RPN	Market Y	Shelf pork product	29 June 2014	AMP,S
MM2.2	Market Z	Butchering pork product	6 June 2014	AMP,S,TE

Notes:^a Slaughterhouse (SLH).^b Ampicillin (AMP); chloramphenicol (C); cefotaxime (CTX); nalidixic acid (NA); streptomycin (S); sulphamethoxazole-trimethoprim (SXT); tetracycline (TE).

In this study, we screened isolates from the pork production process in Northern Thailand for the presence of common, high-risk disease-causing lineages. Using gene-by-gene methods, we characterise a collection of nineteen monophasic *S. Typhimurium* genomes isolated from the pork production chain between 2011–2014 (Patchanee *et al.*, 2016; Tadee *et al.*, 2015). The genetic diversity, presence of antimicrobial resistance, virulence, known plasmid genes and *Salmonella* pathogenicity islands were explored. We identify infiltration of the globally disseminated, multi-drug resistant ST-34 clone at all stages of pork production and assess the pathogenic potential of each isolate. Our findings will aid surveillance and inform agricultural prevention strategies for salmonellosis in the region.

MATERIALS AND METHODS

Monophasic *S. Typhimurium* isolate collection

Monophasic *Salmonella* Typhimurium were isolated during the period of November 2011 through July 2014 in Chiang Mai and Lamphun provinces, as described in the studies of Tadee *et al.* (2015) and Patchanee *et al.* (2016). The studies were conducted under ethical approval reference number R18/2554 by the Animal Care and Use Committee committee of Faculty of Veterinary Medicine, Chiang Mai University (FVM-ACUC). Briefly, samples were collected at three stages of pig production: on the farm ($n = 10$); at the slaughterhouse ($n = 6$) and at retail from local markets and supermarkets ($n = 3$) (Table 1).

Antimicrobial susceptibility assays

All isolates were tested for their susceptibility to antimicrobials using disk diffusion methods following Clinical and Laboratory Standards Institute guidelines (*Clinical & Laboratory Standards Institute (CLSI), 2011*) by the WHO National *Salmonella* and *Shigella* Center, National Institute of Health, Department of Medical Science, Nonthaburi, Thailand. Ten different antimicrobial agents were tested, including ampicillin (AMP) 10 µg, amoxicillin-clavulanic acid (AUG) 20/10 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 ([Table 1](#)).

Whole-genome sequencing

Genomic DNA of 19 monophasic *S. Typhimurium* isolates was extracted, using a QIAamp DNA mini kit (Qiagen, Crawley, UK) and sequenced on an Illumina MiSeq genome sequencer (Illumina, Cambridge, UK). Nextera XT libraries (Illumina, California, USA) were prepared and short paired-end reads (300 bp) trimmed using TRIMMOMATIC (version 0.33; [Bolger, Lohse & Usadel, 2014](#)) and assembled de novo using SPAdes software, with the-careful command (St. Petersburg genome assembler, St. Petersburg, Russia; version 3.10.035) ([Bankevich et al., 2012](#)). The average number of contigs was 400 (range: 187–777) for an average total assembled sequence size of 5.02 Mbp (range: 4.87–5.20) with an average genome coverage of $\times 34$ (range 16–46). The average N50 contig length (L50) was 33,091 bp (range: 10,047–84,409) and the average GC content was 52.2% (range: 52.0–52.5). Short read data are archived on the National Center for Biothechnology Information (NCBI) SRA, associated with BioProject accession [PRJNA573746](#) (individual accession numbers in Supplemental File S1). FASTA files of all contiguous assemblies and [Supplemental Files](#) are archived at the public data repository figshare (<https://doi.org/10.6084/m9.figshare.11305727>).

Multilocus sequence typing

Nucleotide sequences for seven housekeeping gene loci, including *aroC* (chorismate synthase), *dnaN* (DNA polymerase III subunit beta), *hemD* (uroporphyrinogenIII cosynthase), *hisD* (histidinol dehydrogenase), *purE* (phosphoribosyl laminimidazole), *sucA* (alpha-ketoglutarate dehydrogenase) and *thrA* (aspartokinase I/homoserine dehydrogenase) were identified. Alleles and subsequent sequence types (ST) were designated using the web-based MLST 2.0 method at the Center for Genomic Epidemiology (<https://cge.cbs.dtu.dk/services/MLST/>) ([Larsen et al., 2012](#)).

Core genome genealogy

An alignment file was constructed from concatenated gene sequences of all core genes (found in $\geq 95\%$ isolates) from the reference *Salmonella* Typhimurium genome, SO4698-09 (accession# LN999997.1) using MAFFT ([Katoh & Standley, 2013](#)) on a gene-by-gene basis ([Méric et al., 2014](#); [Sheppard, Jolley & Maiden, 2012](#)) (size: 4,166,027 bp; [Table S1](#)). A gene was considered present in a given genome when its sequence aligned to an

SO4698-09 locus with more than 70% sequence identity over at least 50% of sequence length using BLAST. Maximum-likelihood phylogenies were built in IQtree v1.6.8 (Center for Integrative Bioinformatics Vienna, Vienna, Austria) using the GTR + F + I + G4 substitution model. A consensus tree constructed from 1,000 bootstrap trees ([Hoang et al., 2018](#)) and visualised on [microreact](#) ([Argimón et al., 2016](#)).

Whole-genome multilocus sequence typing

Core genome multilocus sequence typing (cgMLST) and pan genome multilocus sequence typing (pgMLST) were performed using BioNumerics version 7.6 (Applied Maths, Ghent, Belgium). A total of 2,978 and 5,022 loci were identified as composing the cgMLST and pgMLST scheme, respectively. Cluster analysis of categorical differences of allelic numbers for cgMLST and pgMLST was applied to generate dendrograms, using the complete linkage algorithms.

Identification of antimicrobial resistance, virulence and plasmid associated genes

Nucleotide sequences of all 19 monophasic *S. Typhimurium* isolates in FASTA format were compared to antimicrobial resistance genes (ARGs), putative virulence factors and known plasmid genes using ABRICATE 0.9.8 (GNU, Boston, MA, USA), using Comprehensive Antibiotic Resistance Database (CARD) ([Alcock et al., 2020](#)), the NCBI ([Feldgarden et al., 2019](#)), ResFinder 3.0 ([Zankari et al., 2012](#)). The virulence factor database (VFDB) ([Chen et al., 2016](#)) and PlasmidFinder 2.1 ([Carattoli et al., 2014](#)) databases. All databases were accessed at 10 September 2019.

Identification of *Salmonella* Pathogenicity Islands

The Center for Genomic Epidemiology was also used to identify SPIs in our *Salmonella* isolates. SPIFinder 1.0 (<https://cge.cbs.dtu.dk/services/SPIFinder-1.0/>) with default settings of 95% threshold over 60% minimum length were used.

RESULTS

Pig isolates from Thailand are related to a globally disseminated clone

All monophasic *S. Typhimurium* isolates were sequenced, genomes assembled and typed using an in silico MLST scheme. Each of the 19 isolates were categorised as sequence type 34 (ST-34), with the allelic profile: *aroC_10/dnaN_19/hemD_12/hisD_9/purE_5/sucA_9/thrA_2*. Isolate genomes were mapped to the *S. Typhimurium* ST-34 reference, SO4698-09 and homologues of all core genes ([Table S2](#)) aligned and used to construct a maximum-likelihood phylogenetic tree ([Fig. 1](#)). Isolates were closely related and the sum of all internal branch lengths represented less than 5% of the tree length, indicating that all isolates were closely related to the globally disseminated, livestock-associated ST-34 clone.

Extended whole genome typing analysis (wgMLST) of the 19 monophasic *S. Typhimurium* genomes was performed for core- (cgMLST; 2,978 genes present in 100% of isolates) and pan- (pgMLST; 5,022 genes) genomes. All isolates were closely related, with a maximum

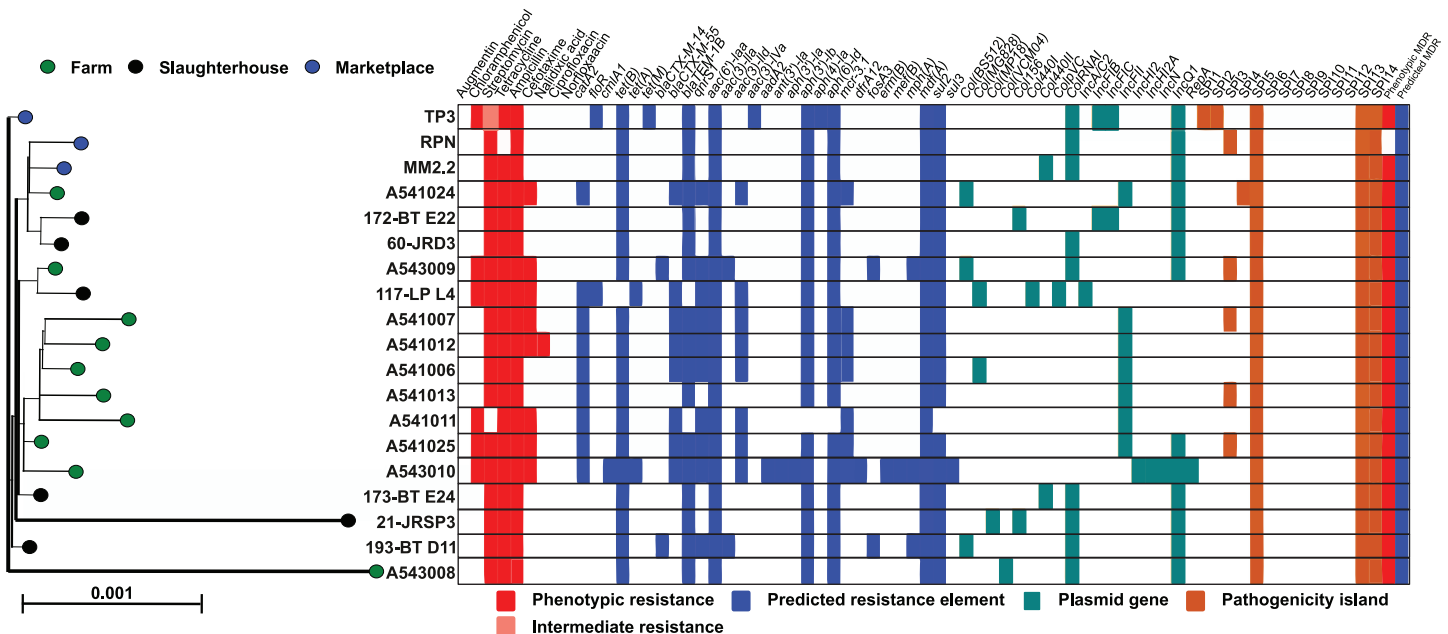


Figure 1 Genetic relatedness of *S. enterica* serovar Typhimurium isolates. Genes present in 95% or more of the isolate genomes were aligned (4,166,027 bp) and a maximum-likelihood phylogeny was reconstructed in IQ-TREE with the generalised time reversible substitution model and ultrafast bootstrapping. Scale bar represents genetic distance of 0.001. Each leaf represents a single isolate and is coloured according to sampling location: farm (green), slaughterhouse (black) and retail marketplace (blue). Alongside the tree is a matrix highlighting phenotypic resistance (red), intermediate resistance (light red), presence of predicted AMR genes (blue), plasmid genes (yellow) and presence of *Salmonella* Pathogenicity Islands (orange). [Full-size !\[\]\(1679558f37f6db0dd8360a2a7e913e90_img.jpg\) DOI: 10.7717/peerj.9700/fig-1](https://doi.org/10.7717/peerj.9700/fig-1)

130 and 170 locus differences between isolates, for cgMLST and pgMLST analyses respectively. Isolates A541011 and A541013 were the most closely related isolates and were collected from the same farm (PD farm), on the same sampling date (15 November 2011) and only differed in 18 core loci. When accessory genes were included in the pan-genome analysis, isolates A541006 and A541012 were the most closely related, with 26 loci differences. Visual comparison of the core- and pan-genome MLST dendrograms demonstrates similarity in the clustering of isolates (Fig. S1).

Clonal isolates shared different ARGs

The genome sequences of all 19 monophasic *S. Typhimurium* isolates were compared using ABRICATE 0.9.8 and known antibiotic resistance genes and alleles from CARD, ResFinder and NCBI databases (Table S3). As there is redundancy in these antibiotic resistance gene sequence databases, we show only results from the ResFinder comparison (Fig. 1). Twenty-five resistance genes were identified, from ten antibiotic classes (Table 2). Eight detected genes (8/25; 32%) were grouped in aminoglycoside classes. All monophasic *S. Typhimurium* genomes (19/19; 100.00%) harboured aminoglycoside and beta-lactam class resistance genes. Sulphonamide and tetracycline resistance genes were the next most prevalent (18/19; 94.73%), followed by fluoroquinolone (10/19; 52.63%) and phenicol (7/19; 36.84%). Fusidic acid, glycopeptide, nitroimidazole, oxazolidinone and rifampicin associated-resistance genes were not detected in any of the genomes. Putative resistance genes were also detected for colistin (7/19; 36.84%), phosphomycin (2/19; 10.52%)

Table 2 Frequency of resistance genes detected from 19 monophasic *Salmonella* Typhimurium isolated from pig production in Chiang Mai and Lamphun.

Class	Resistance gene	No. of strains carried the gene (%)	No. of strains carried gene grouped in the class (%)
Aminoglycoside	<i>addA1</i>	1 (5.26)	19 (100.00)
	<i>addA2</i>	1 (5.26)	
	<i>aac(3)-IIa</i>	5 (26.32)	
	<i>acc(3)-IVa</i>	1 (5.26)	
	<i>acc(3)-IIId</i>	6 (31.58)	
	<i>aph(3)-Ib</i>	18 (94.97)	
	<i>aph(4)-Ia</i>	1 (5.26)	
	<i>aph(6)-Id</i>	18 (94.74)	
Beta-lactam	<i>blaTEM-1B</i>	17 (89.47)	19 (100.00)
	<i>blaCTX-M-14</i>	2 (10.53)	
	<i>blaCTX-M-55</i>	8 (42.11)	
Fluoroquinolone	<i>qnrS1</i>	10 (52.63)	10 (52.63)
Phenicol	<i>catA2</i>	9 (47.37)	10 (52.63)
	<i>cmlA1</i>	1 (5.26)	
	<i>floR</i>	2 (10.53)	
Sulphonamide	<i>sul2</i>	18 (94.74)	18 (94.74)
	<i>sul3</i>	1 (5.26)	
Tetracycline	<i>tet(A)</i>	2 (10.53)	18 (94.74)
	<i>tet(B)</i>	17 (89.47)	
	<i>tet(M)</i>	1 (5.26)	
Trimethoprim	<i>dfrA12</i>	1 (5.26)	1 (5.26)
Fosfomycin	<i>fosA4</i>	2 (10.53)	2 (10.53)
MLS*	<i>ermB</i>	1 (5.26)	3 (15.79)
	<i>mphA</i>	3 (15.79)	
Colistin	<i>mcr-3.1</i>	7 (36.84)	7 (36.84)

Note:

* MLS, macrolide-lincosamide-streptogramin B.

and macrolide-lincosamide-streptogramin B (3/19; 15.78%), although no phenotypic susceptibility tests were performed to support these results.

Predicted resistance generally matched the phenotypic susceptibility profiles (Table 3). Nearly half of the antimicrobial classes tested (2/5; beta-lactam and tetracycline) were 100% concordant. In other comparisons, some isolates were predicted to be resistant, but no phenotypic resistance was observed ($n = 2$ aminoglycoside; $n = 9$ fluoroquinolone; $n = 5$ phenicol). Conversely, one isolate was phenicol phenotypically resistant, but no antimicrobial resistance determinant was identified.

Agricultural reservoir isolates contribute to public health risk

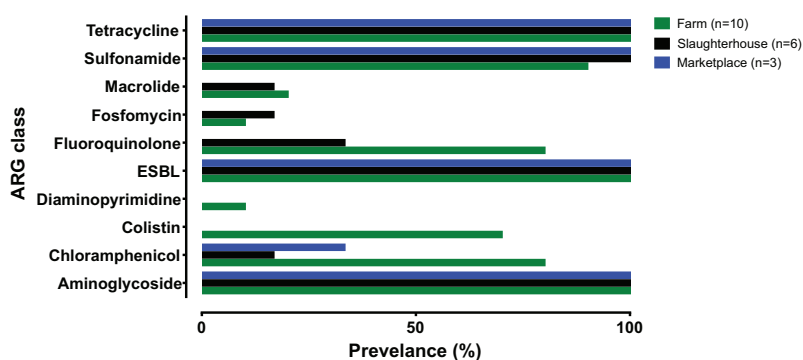
Fewer ARG determinants were found in the isolates as pigs passed through the production process (farm: $n = 10$, mean = 11.8; slaughterhouse $n = 6$, mean = 8.5; marketplace $n = 3$, mean = 8.3), although with only a small number of samples strong statistical

Table 3 Comparison of antimicrobial resistance characteristics of monophasic *Salmonella* Typhimurium ($n = 19$) isolated from pig production in Chiang Mai and Lamphun.

Antimicrobial class	Strain characteristic ^a				Accuracy ^b
	P+/G+	P+/G-	P-/G+	P-/G-	
Aminoglycoside	17	–	2	–	0.89
Beta-lactam	19	–	–	–	1
Fluoroquinolone	1	–	9	9	0.53
Phenicol	5	1	5	8	0.68
Tetracycline	18	–	–	1	1

Notes:

Not available (na).

^a Phenotype (P); genotype (G); presence (+); absence (–).^b The value is calculated by $(P+/G+) + (P-/G-)/n$.**Figure 2** Summary of antimicrobial resistance genes (ARGs) identified, grouped by antibiotic class and collection source. Farm (green), slaughterhouse (black) and retail marketplace (blue).

Full-size DOI: 10.7717/peerj.9700/fig-2

associations cannot be made. Samples collected from retail pork products were not predicted to confer resistance to macrolides, fosfomycin, fluoroquinolone, diaminopyrimidine or colistin (Fig. 2). Typically, isolates collected from farm animals contained ARGs conferring resistance to a broader range of antimicrobials. Comparison with the Plasmid finder database identified the ColRNAI plasmid (accession: DQ298019) in all marketplace isolates (3/3; 100%), nearly two-thirds (4/6; 66%) of slaughterhouse isolates and only a fifth of farm isolates (2/10; 20%) (Fig. 1). In addition, the IncQ1 plasmid (accession: M28829.1) was identified in multiple samples (13/19; 68%).

Identification of *Salmonella* Pathogenicity Island genes

ABRICATE was also used to identify known virulence genes in our monophasic *S. Typhimurium* isolates with Virulence finder database (VFDB). In total, 137 different virulence genes were identified, including those from several *Salmonella* Pathogenicity Islands (SPIs) (Table S3). Seven out of 14 previously described SPIs were detected (Fig. 1A), supported by additional investigation of previously described SPIs using the SPIFinder 1.0 database. Isolates carried three to six SPIs, with more than half (12/19) carrying three islands. The TP3 isolate obtained from a retail pork sample at a local market

carried six previously described SPIs (SPI-1, SPI-2, SPI-3, SPI-5, SPI-13 and SPI-14) and eleven antibiotic resistance gene determinants from six different antibiotic classes. All the monophasic *S. Typhimurium* isolates harboured SPI-5, SPI-13 and SPI-14 (100.00%; 19/19 strains), followed by SPI-3, which was carried by nearly one-third of them (31.57%; 6/19 strains).

The large *Salmonella* genomic island, SGI-4 (up to 87 genes) is often found in ST-34 clones from pigs. Gene-by-gene comparison of our isolates with the *Typhimurium* reference strain SO4698-09 identified many of the genes linked to carriage of the SGI-4 element (Table S3), including the mercury resistance genes *merEDACPTR*, which are found near an arsenate resistance operon (*aseR*, *arsD*, *arsA1*, *arsB*, arsenate reductase, bar N-acetyltransferase, *arsA2*, *arsD2*) and silver-copper resistance element (*silESRCFBAGP pcoG silE pcoABCDRSE2*). Variation has been observed in the SGI-4 genomic island, which has been found to contain up to 87 loci, with 48 described as core. In our collection, at least 84 loci (97%) of the SGI-4 island were identified in all isolates (Table S3).

DISCUSSION

Globalisation and industrialisation of food production has contributed to the emergence of opportunistic pandemic clones (Anderson, 2010). In this study, all of the 19 monophasic *S. Typhimurium* strains collected from different stages of the pig production process in Northern Thailand were found belong to ST-34, a globally disseminated, multidrug resistant clone closely associated with industrial livestock infections and is often implicated in human food-borne outbreaks (Gymoese et al., 2017; Luo et al., 2019; Zheng & Feng, 2019). Isolates were collected from different stages of the pork production process in Northern Thailand, all of which were found to have been infiltrated by the ST-34 pandemic clone.

We employed a genome-wide, gene-by-gene approach, supported by curated online reference data sets, which provide a standardised MLST nomenclature and the potential to analyse large datasets without specialist bioinformatics expertise or resources (Maiden et al., 2012). Isolates collected from the same place had 18 and 26 loci differences, in core and pan-genome MLST typing schemes, respectively (Fig. S1). Some evidence of heterogeneity was found within the herd, although the sum of all internal branch lengths represented was less than 5% of the core genome maximum-likelihood tree length (Fig. 1), indicating that all isolates were closely related.

Rising AMR in bacteria is a WHO priority, particularly for gastrointestinal pathogens such as *Salmonella* (World Health Organization (WHO), 2015). The contribution of resistance acquired by livestock associated pathogenic strains on human infection is unclear (Holman & Chenier, 2015; Tanwar et al., 2014; Schwarz, Kehrenberg & Walsh, 2001). Antibiotic use as growth promoters has been banned in European agriculture since 2006, however quinolones and tetracyclines are still available for treatment of livestock (World Health Organization (WHO), 2015). Regulation is less stringent in Thailand, and

nearly all the 19 monophasic *S. Typhimurium* isolates carried resistance elements belonging to aminoglycoside ($n = 19$), beta-lactam ($n = 19$), tetracycline ($n = 18$) and sulphonamide ($n = 18$) classes which are among the most widely used in industrial pig farming (Van Rennings *et al.*, 2015). Continuous administration at sub-therapeutic level or use as a growth promoter can select for resistance determinants (Holman & Chenier, 2015), and as suggested previously, streptomycin, ampicillin, sulphonamide, tetracycline and similar class antimicrobial are not recommended for clinical salmonellosis treatment (Campioni, Moratto Bergamini & Falcao, 2012; Patchanee *et al.*, 2016; Steeve, John & Dowling, 2013). Several of the isolates collected from farm animals contained colistin resistance elements (7/10, 70%), where colistin may have been used, however these were not found in isolates from later stages of the production process.

Differences were observed between genotype prediction and laboratory phenotype, for example no known antimicrobial resistance determinant could be identified for one isolate that was phenotypically resistant to chloramphenicol (Fig. 1; Table 3). This finding suggests that there may be additional chloramphenicol resistance genes not yet identified or available in the databases. Variation in gene expression levels or synergistic effects among different resistance genes can confound DNA-based predictions and warrants further study (Neuert *et al.*, 2018). Carriage of resistance phenotype and their resistance genes are not routinely tested for some antibiotics (as in our case). This means that isolates carrying genes that can contribute to antibiotic resistance will be missed (Rosengren, Waldner & Reid-Smith, 2009). Practical and financial constraints can limit the number of selected antimicrobial types that are tested in the laboratory, in silico genotypic identification offers a potential solution to this, and when combined with machine-learning approaches can be highly accurate (Bradley *et al.*, 2015; Nguyen *et al.*, 2019).

Potential transmission of AMR bacteria between agricultural animals and humans is also a concern, but controversy remains interpreting what constitutes the spread of resistance (Boerlin & Reid-Smith, 2008; Mourkas *et al.*, 2019). Resistance can be transmitted through the food production chain to infect humans, or via recombination and horizontal gene transfer (Yahara *et al.*, 2017). In *Salmonella*, virulence-associated genes are often mobilised on pathogenicity islands (SPIs) or plasmids and can mediate interactions with the host (Amavisit *et al.*, 2003; Marcus *et al.*, 2000). Only a single isolate in our collection contained SPI-1 and SPI-2, which encode type III protein secretion systems required for intestinal invasion (Lou *et al.*, 2019). The SPI-3 island found in five of our isolates harbours the *mgtC* gene required for intramacrophage survival (Blanc-Potard *et al.*, 1999). Pathogenicity island-4 (SPI-4), found in one of our isolates, carries a type I secretion apparatus contributing to the colonisation of bovine intestines (Kiss, Morgan & Nagy, 2007). Three SPIs were found in all 19 monophasic *S. Typhimurium* (SPI-5, SPI-13 and SPI-14). SPI-5 is often associated with enteropathogenesis, mediating the inflammation and chloride secretion that characterise the enteric phase of disease (Marcus *et al.*, 2000). SPI-13 is required for efficient utilisation of D-glucuronic acid (DGA) and tyramine (TYR) as sole energy sources (Elder *et al.*, 2018). SPI-14 harbours eight open reading frames (ORFs) encoding putative cytoplasmic proteins and is significant during host invasion and colonisation (Jiang *et al.*, 2017).

CONCLUSIONS

In this study, we demonstrate that samples tested from multiple stages of the Thai pork production process have been contaminated by the globally disseminated, multi-drug resistant ST-34 clone of monophasic *S. Typhimurium*. Isolates show differences in virulence and antimicrobial resistance profiles, likely affected by acquisition and loss of accessory elements through horizontal gene transfer, mediated by SPIs and plasmids. These clones pose a tangible public health risk, as evidenced by the TP3 strain obtained from a retail market which harbours the SPI-1 pathogenicity island that is important for invasion of host cells and induction of macrophage apoptosis (*Amavisit et al., 2003*). This emphasises the importance of maintaining hygiene standards and all pork products should be cooked well. Microbial surveillance will help guide intervention measures and provide a reminder that sanitation and hygienic practices at all production levels should be prioritised.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Prapas Patchanee conceived and designed the experiments, analysed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Prawitchaya Tanamai conceived and designed the experiments, performed the experiments, analysed the data, prepared figures and/or tables, and approved the final draft.

- Phacharaporn Tadee performed the experiments, prepared figures and/or tables, and approved the final draft.
- Matthew D. Hitchings performed the experiments, prepared figures and/or tables, and approved the final draft.
- Jessica K. Calland analysed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Samuel K. Sheppard conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Dethaloun Meunsene performed the experiments, prepared figures and/or tables, and approved the final draft.
- Ben Pascoe conceived and designed the experiments, analysed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Pakpoom Tadee conceived and designed the experiments, analysed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

Ethics

The following information was supplied relating to ethical approvals (i.e. approving body and any reference numbers):

The study was conducted under ethical approval granted by the Animal Care and Use Committee committee of Faculty of Veterinary Medicine, Chiang Mai University (FVM-ACUC) (R18/2554).

DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

Short read data are available at NCBI SRA: [PRJNA573746](https://www.ncbi.nlm.nih.gov/sra/PRJNA573746).

Data Availability

The following information was supplied regarding data availability:

All contiguous assemblies are available at Figshare: Pascoe, Ben (2019): Salmonella from the pork production process in Thailand. figshare. Dataset. DOI [10.6084/m9.figshare.9894272.v1](https://doi.org/10.6084/m9.figshare.9894272.v1).

Supplemental Information

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