CHAPTER 5

ANTIMICROBIAL RESISTANCE GENE AND SURVIVAL ABILITY CHARACTERIZATION OF *Salmonella* USING WHOLE GENOME SEQUENCING AND GENOME WIDE ASSOCIATION STUDY

5.1 Abstract

Foodborne infection with *Salmonella* and antimicrobial resistant (AMR) *Salmonella* are a serious public health risk, economic security threat and are widespread in developed and developing countries through the food production chain. Furthermore, the persistence of this pathogen can be found along food production chain and it's hard to eradicate. The objectives of this study are in order to find out genetic diversity of *Salmonella* spp. and antibiotic resistant genes and comparison of genetic diversity of WGS result and pulsotypes identified by previous study and to investigate the survival abilities of Salmonella spp. though food production chain, Chiang Mai and Lamphun, Thailand. In total of 124 *Salmonella* isolates were recovered from the pork production chain in Chiang Mai and Lamphun, Thailand over four years were sequenced by an Illumina MiSeq. Resistance gene testing used the tools in the form of the Comprehensive Antibiotic Research Database. Genome wide association study was done to find out the gene associated with survival of *Salmonella spp.* in swine production chain. The contamination of *Salmonella* results were thoroughly found along swine production chain revealed by nucleotide difference tree which make whole genome sequencing offers greater resolution than the gold-standard PFGE typing method. Furthermore, this tool is useful for prediction of antimicrobial resistance which help for surveillance the emerging of antimicrobial resistance pathogen.

Genome wide study was found 37 genes associated with survival of this pathogen and can be confirmed why this pathogen is persisted and very hard to eradicate in swine production chain. Good management of antiseptic and antimicrobial usage is very important to concern.

5.2 Introduction

Foodborne infection with *Salmonella* is responsible for 93.8 million cases of gastroenteritis reported each year and 155,000 deaths worldwide [6, 48, 99-100]. The most common human infection source is consumption of contaminated pork products [58]. Intensification of agricultural practices have encouraged the emergence of zoonotic disease and is a global health risk and economic security threat. Feacal contamination of the pork production process gives Salmonella opportunity to contaminate the food chain, from farm to fork [35, 60], as reported in Thailand [44, 58]. Despite legislature to control the use of antimicrobial agents in livestock feeds there is little control of these agents in SE Asia. Antimicrobial resistant (AMR) Salmonella are a serious public health risk and are widespread in developed and developing countries through the food production chain [101-102]. *Salmonella* have shown resistance to many antimicrobial agents, including Aminoglycosides, Tetracycline, Chloramphenicol, Sulfamethoxazole-Trimethoprim, Sulfonamide, Fluoroquinolones and β-lactamases [103-105]. Extended spectrum β-lactamase (ESBL) producing *Salmonella* pose a clinical problem which are emerging globally [106]. However, AMR prediction can improve surveillance and inform effective treatment. Identification of AMR genes from genome sequence data have been used to improve global antimicrobial surveillance, for instance in *Staphylococcus aureus*, *Mycobacterium tuberculosis* [107,108], *Escherichia coli* [109], but as yet no report in *Salmonella*.

Salmonella can be persisted in food production chain or the environment and can contaminated the foods via any pathways that reveal the variety of ecosystems that make up our food supply. In previous study of [35, 60] were found some persistence strains and cross contamination along swine production chain. Biofilm formation might be play role on this situation. Several studies have shown that *Salmonella* can attach and

form biofilms on surfaces found in food-processing factory including plastic, cement, and stainless steel [110-111] according to hard to kill them by only one kind of disinfectant. Biofilm forming in food-processing environment are special potential to persistent source of microbial contamination and can easily be spread along the food production chain as a consequence of inappropriate cleaning [112]. In addition, the formation of multicellular biofilms is an ancient adaptation which that structure system is essential for bacteria to survive in environment [113]. Furthermore, antimicrobial resistance and virulence traits may represent a survival advantage to the microorganism [114]. That are virulence traits are important for organism to overcome host defend system, and emerging antimicrobial resistance is help pathogens to overcome antimicrobial therapies and to adapt and survive in competitive and demanding environments [115]. This is due to the role of disease transmission which increases the food safety risk [113, 116].

Whole genome sequencing (WGS) offers greater resolution than the gold-standard PFGE typing method [34] and facilitates the study of genetic diversity [36], understanding bacterial evolution, outbreaks, transmission events and disease surveillance [37]. They can be tools for consumer protection and can be refer to make stand method controlling and protecting of *Salmonella* contamination and control antimicrobial resistance in swine production chain and healthcare [117]. However, the previous studies of *Salmonella* genetic diversity were not quite completely explain as well as the some association of genes or loci between the phenotype are related [35,60]. Interestingly, there is a tool in the form of the Comprehensive Antibiotic Research Database (CARD; http://arpcard.mcmaster.ca) to provide quickly identify antibiotic resistance genes in new unannotated genome sequences. The card is collected more than 1,600 antibiotic genes and all are the active and ongoing sequences that available in Genbank [118].

Genome-wide association studies (GWASs) are the study of testing large numbers of genetic variants such as single nucleotide polymorphisms (SNPs) or insertions or deletions (indels) or k-mer which associations with interested phenotypes by logistic regression statistically [39, 119]. Genome-wide association study are becoming

important approach for eukaryotic geneticists which identified genetic polymorphisms which are related to inherited diseases [120]. The first successful GWAS in bacteria was developed in the study of [39]. Bacterial GWAS is a brand new bacteriology to have deepened the understanding of phenotype variation [120]. However, bacteria GWAS is a bit complicate because of their strong structuring into distinct strains and substantial linkage disequilibrium through the genome [121]. Nevertheless, bacterial GWAS is becoming an increasingly powerful methodology and the study of [122] can have a successful associated mapping to applied in genotyping bacterial strategies. In this study, These techniques can be improved the understanding of how genetic variation in natural bacterial populations may influence their ecology [113]. This is the first study of GWAS applied in veterinary medicine filed in Thailand.

The objectives of this study are in order to find out genetic diversity of *Salmonella* spp. and antibiotic resistant genes and comparison of genetic diversity of WGS result and pulsotypes identified by previous study [60] and to investigate the survival abilities of Salmonella spp. though food production chain, Chiang Mai and Lamphun, Thailand.

5.3 Materials and methods

5.3.1 *Isolates*

One hundred and twenty-four *Salmonella* isolates were recovered from the pork production chain in Chiang Mai and Lamphun, Thailand over four years (2011-2014; Table 5.1). Strains were isolated from pig faeces, skin, carcass, slaughterhouse workers and environmental samples. All isolates were subject to antimicrobial susceptibility assay. Ten antimicrobial agents were determined including ampicillin (AMP) 10 μg, amoxicillin-clavulanic acid (AUG) 30 μg, sulfamethoxazole-trimethoprim (SXT) 25 μg, ciprofloxacin (CIP) 5 μg, chloramphenicol (C) 30 μg, streptomycin (S) 30 μg, nalidixic acid (NA) 30 μg, norfloxacin (NOR) 10 μg, cefotaxime (CTX) 30 μg and tetracycline (TE) 30 μg. Serotyping and antimicrobial susceptibility testing in this study were performed by WHO National Salmonella and Shigella Center Laboratory (NSSC), Nonthaburi, Thailand. Finally, 684 global collections downloaded from National Center

for Biotechnology Information (NCBI) were used to analyse for population structure and phylogenies

Table 5.1 Number of *Salmonella* strain tested from swine production chain (farm to market) in Chiang Mai and Lamphun province, Thailand recovered 2011-2014

Salmonella serotypes	Number of isolates			
	Farm	Slaughterhouse	Market	Total
S. Agona	0	0	1	1
S. Anatum	0	0	3	3
S. Covallis	0	0	1	1
S. Give	3	2	2	7
S. I.4,5,12 : i : -	0	0	3	3
S. Kedougou	0	0	3	3
S. Krefeld	0	0	2	2
S. Lexington	0	0	1	1
S. Newport	0	0	1	1
S. Panama	2	4	0	6
S. Rissen	12	23	11	46
S. Stanley	3	10	0	13
S. Typhimurium	9	2	0	11
S. Weltevreden	2	4	2	8
S. Yoruba	0	0	1	1
S.Corvallis	0	0	1	1
S.I.4,5,12:i:-	10	6	0	16
Total	41	51	32	124

5.3.2 *Genome Sequencing*

124 Thai isolates were extracted by QIAamp DNA mini kit for sequencing using an Illumina MiSeq. Illumina Nextera XT DNA sample preparation kit was used to construct libraries. High coverage short reads were assembled de novo using SPAdes software [123]. All sequence data are stored in Bacterial Isolate Genome Sequence Database (BIGSdb) software and analysed for population structure and phylogenies [39,

124-125]. All whole genome sequencing data were aligned by using MAFF *Salmonella enterica* subsp. enterica serovar Typhimurium str. LT2 chromosome, complete genome accession number: NC_003197 is the reference of this analysis. Phylogenetic trees were generated by MEGA version 6.

5.3.3 *Identification of putative AMR genes*

Resistance gene testing in this study used the tools in the form of the Comprehensive Antibiotic Research Database (CARD; http://arpcard.mcmaster.ca) [120] with Gene-by-gene approach by Bigsdb genome comparator tool.

5.3.4 Genetic diversity comparison among PFGE and WGS

Some of *Salmonella* strains (82 isolates) were done PFGE from previous study [60] and compared genetic diversity with WGS. Simpson's Index of diversity is the diversity measures in this study. In addition, the adjusted Rand coefficient and the Wallace coefficient were calculated to determine the concordance of the two typing techniques and the relative ability of the two techniques to predict directional information [65]. Diversity and partition congruence coefficients calculation are in online tool via http://www.comparingpartitions.info/index.php?link=Tool

5.3.5 *Biofilm formation*

Briefly, 100 µl of LB Broth was inoculated with 30 µl aliquots of overnight culture (OD595 between 1.0 and 1.5) in a 96-well plate. Plates were incubated at 37°C for 24 hr . Culture medium was removed and the wells washed with PBS. Plates were air-dried and then stained with 130 µl of 0.1% (w/v) crystal violet for 30 min. Excess stain was removed and the wells washed with PBS, adhered bacteria were air-dried and add 130 µl ethanol:acetone (70:30 w:w) and incubation for 10 min in room temperature. OD595 were measured after the bound dye was dissolved using ethanol:acetone. The result was calculated by subtracting the median OD595 of the three parallels of the control from the median OD595 of the three parallels of sample. (BMG Omega)[113].

5.3.6 *Genome mapping*

Genome wide association study was followed the method by the study of [39] using 30 bp `word' searching base on biofilm formation.

5.4 Results

5.4.1 *Population structure based on Whole genome sequencing*

WGS generated profiles of 124 *Salmonella* isolates and compared them to 684 global collections. Thai *Salmonella* isolates were not segregated from other strains (Figure 5.1). Considering in 124 *Salmonella* strains in swine production chain, Thailand, There are the relations between many strains from farm to market (Figure 5.3).

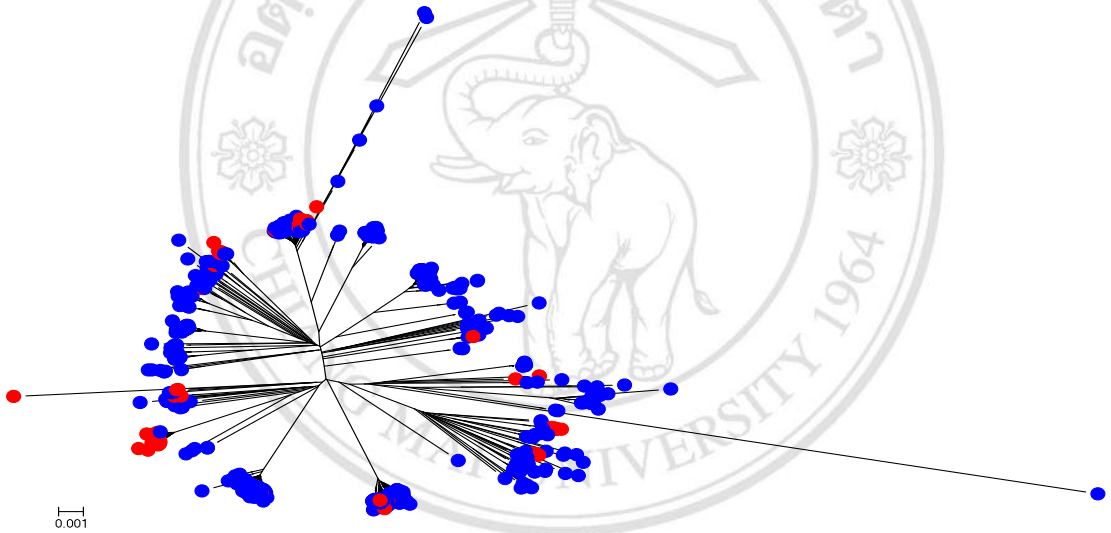

Figure 5.1 Population structure of 124 Thai *Salmonella* isolates (red) compared to global *Salmonella* collection (Blue)

Figure 5.2 Whole genome typing of 124 *Salmonella* strains from swine production chain, Thailand showing by Nucleotide difference tree

5.4.2 *Core/whole genome phylogenies correlate with previous typing methods*

Some of *Salmonella* isolates (82 isolates) were done PFGE from previous study) and compared genetic diversity with WGS. Simpson's Index of diversity of PFGE and WGS were 0.919 and 0.993, respectively. The ability of type of PFGE can be typed into 7 clusters (A-G). However, WGS can be typed into 8 clusters (A-H) Figure 5.2. In addition, the Adjusted Rand coefficient was 0.057. The Wallace coefficient of PFGE to WGS was 0.037 and the Wallace coefficient of WGS to PFGE was 0.455 (Table 5.2)

Table 5.2 Simpson's diversity and coefficient indexes comparing PFGE and WGS methods

Typing method	Simpson's index of diversity (95% CI)	Adjusted Rand coefficient (95% CI)		Wallace coefficient (95% CI)	
		PFGE	WGS	PFGE	WGS
PFGE	0.919 (0.889-0.949)	1.000 (1.000-1.000)		1.000 (1.000-1.000)	0.031[a] (0.000-0.075)
WGS	0.993 (0.991-0.996)	0.057 (0.003-0.110)	1.000 (1.000-1.000)	0.407[b] (0.235-0.578)	1.000 (1.000-1.000)

[a]Wallace coefficient of PFGE to WGS

[b]Wallace coefficient of WGS to PFGE

5.4.3 *Efficient prediction of antimicrobial resistance based on sequence data*

In this study found the relationship between antibiotic resistance phenotype and genotype. Some strain was found no relationship between phenotype and genotype, that were shown all susceptible but there were resistance gene inside the strain.

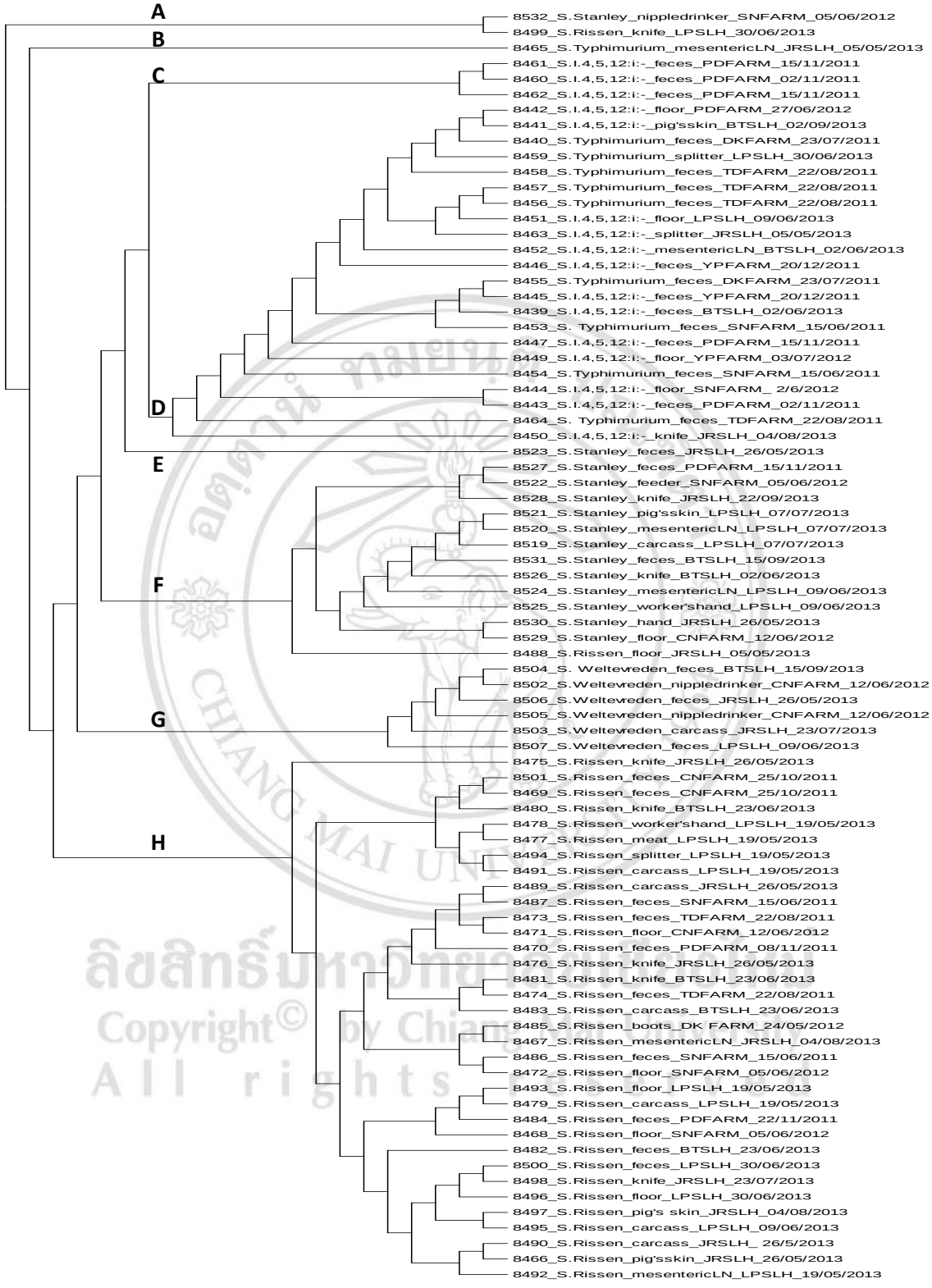

Figure 5.3 Whole genome typing of 82 S*almonella* strain showing by Nucleotide difference tree.

Interestingly, not only antibiotic resistance, the study was also found antiseptic resistance genes and anti-Copper genes. (Table 5.3). Significance level and Odds ratio, P-value among antimicrobial resistance gene and phenotype was shown in Table 5.4. The statically significant (P<0.05) was shown in Chloramphenicol, Streptomycin, Ampicillin, Tetracycline and Sulfamethoxazole-Trimethoprim. The statically was not significant (P>0.05) was shown in Nalidixic acid.

5.4.4 *High levels of ESBL resistance predicted in Thailand isolates*

blaTEM-1, *blaCTX-M-14*, *blaCTX-M-18*, *blaCTX-M-55*, *bla CTX-M-57*, blatem precursor confer extended-spectrum β-lactamases (ESBLs) were found 73.8%. Tetracycline resistance gene was found the highest prevalence with 85.7%. (Table 5.4).

5.4.5 Genome wide association study

469 genes containing associated elements with biofilm formation.There were 37 genes associated with survival of *Salmonella* spp. in swine production chain (Figure5.4-5.5).

Table 5.3 Characterized of Antimicrobial resistance phenotyping and genotyping of *Salmonella* spp. from food production chain (farm-market), Chiang Mai-Lamphun, Thailand.

ID	Antimicrobial resistance	
	Phenotype	**Gene**
8425	S	*SeAg_B4524/soxS*
8426	AMP,SXT,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR /sul3/dfrA12/aadA1/blatem precursor/aadA2/qacEdelta 1/soxS*
8427	AMP,SXT,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR /sul3/dfrA12/aadA1/blatem precursor /aadA2/qacEdelta 1/soxS*
8428	AMP,SXT,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/aadA2/qacEdelta 1/soxS*
8429	AMP,C,S,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/aph6/VCD_003731/blatem precursor /aacC4/HCM1.222/TetA/aph(4)/strB/STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*
8430	AMP,S	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/aph6/VCD_003731/blatem precursor/ HCM1.222/strB/ STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580/*
8431	AMP,S,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/aph6//VCD_003731/blatem precursor/strA/HCM1.222 /TetA/strB/soxS*
8432	S	
8433	ALL SUSCEPTIBLE	*TetR/BlaTEM-1/sul3/blatem precursor /aadA/cmlA1/aadA2*
8434	AMP,C,S,TE	*TetR/BlaTEM-1/sul3/blatem precursor /aadA/cmlA1/aadA2*
8435	AMP,S,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/aph6/VCD_003731/blatem precursor/strA/HCM1.222/TetA/strB/STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*
8436	AMP,S,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/aph6/VCD_003731/blatem precursor /strB/HCM1.222/TetA/strB/soxS*
8437	AMP,SXT,C,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/qnrS/sul3/dfrA12/qnr/aadA1/dihydropteroate synthase/blatem precursor/ECL_03814/aadA2/qacEdelta 1/soxS/sul2*

Table 5.3 (continued)

ID	Antimicrobial resistance	
	Phenotype	**Gene**
8438	ALL SUSCEPTIBLE	
8439	AMP,S,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/aph6/VCD_003731/blatem precursor/ HCM1.222/TetA/strB/STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*
8440	AMP,C,S,CTX,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/blaCTX-M-55/bla CTX-M-57/aph6/qnr/blatem precursor/TetA/strB/STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*
8441	AMP,S,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/blaCTX-M-14/blaCTX-M-18/qnrS/mph(A)/aph6/mphA/VCD_003731/blatem precursor/ /HCM1.222/TetA/strB/ STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580/ mph(A)/ FosA4*
8442	AMP,C,S,CTX,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/blaCTX-M-14/blaCTX-M-18/qnrS/mph(A)/aph6/VCD_003731/blatem precursor/ HCM1.222/TetA/strB/STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580/FosA4*
8443	AMP,S,CTX,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/blaCTX-M-55/bla CTX-M-57/qnrS/aph6/qnr/VCD_003731/blatem precursor/blaTEM1b/HCM1.222/TetA/strB/STM0352.S/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*
8444	AMP,S,TE	*TetR/SeAg_B4524/strA/strB/aph6/VCD_003731/HCM1.222/TetA/strB/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*

Table 5.3 (continued)

ID	Antimicrobial resistance	
	Phenotype	**Gene**
8445	AMP,S,CTX,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/blaCTX-M-55/bla CTX-M-57/qnrS/aph6/VCD_003731/blatem precursor/HCM1.222/TetA/strB/STM0352.S/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*
8446	AMP,C,S,CTX,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/blaCTX-M-55/bla CTX-M-57/qnrS/aph6/VCD_003731/blatem precurso/HCM1.222/TetA/strB/STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*
8447	AMP,S,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/aph6/VCD_003731/blatem precursor/HCM1.222/TetA/strB/STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*
8448	AMP,C,CTX,S,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/blaCTX-M-55/bla CTX-M-57/qnrS/aph6/qnr/dihydropteroate synthase/blatem precursor/ECL_03814/ TetA/strB/sulII/STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*
8449	AMP,SXT,C,S,CTX,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/aadA1a/blaCTX-M-55/bla CTX-M57/qnrS/sul3/mph(A)/aph6/qnr/mphA/aadA1/VCD_003731/blatem precursor /HCM1.222/ STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580/cmlA1*
8450	AMP,S,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/aph6/VCD_003731/blatem precursor/blaTEM1b/HCM1.222/TetA/strB/STM0352.S/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580/strA/strB/*

Table 5.3 (continued)

ID	Antimicrobial resistance	
	Phenotype	**Gene**
8451	AMP,C,S,CTX,TE	*SeAg_B4524/strA/strB/blaCTX-M-55/bla CTX-M-57/qnrS/aph6/VCD_003731/dihydropteroate synthase/ECL_03814/sulII/strB/STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*
8452	AMP,S,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/aph6/ VCD_003731/blatem precursor/HCM1.222/TetA /STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*
8453	AMP,S,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/aph6/VCD_003731/blatem precursor/strA/HCM1.222/TetA/strB/STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/STM0354/STM1619/STM0580*
8454	AMP,S,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/aph6/ /VCD_003731/blatem precursor/HCM1.222/TetA/strB/STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*
8455	AMP,S,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/aph6/ VCD_003731/blatem precursor/HCM1.222/TetA/strB/STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*
8456	AMP,S,TE	*SeAg_B4524/sul1/ACICU_00228/sulI/ABAYE3616/blaTEM/ aac3-VI/sulI/qacEdelta 1/STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580/hypothetical protein. ORF4*
8457	AMP,S,TE	*TetR/SeAg_B4524/strA/strB/sul1/aadA1a/strA_or_rpsL/aph6/ACICU_00228/ABAYE3616/dihydropteroate synthase/ECL_03814/blaTEM/ aac3-VI/sulI/qacEdelta 1/ STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580/Hypothetical protein. ORF4*

Table 5.3 (continued)

ID	Antimicrobial resistance	
	Phenotype	**Gene**
8458	AMP,S,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/aph6/VCD_003731/blatem precursor/HCM1.222/strA/TetA/strB/STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*
8459	AMP,S,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/VCD_003731/blatem precursor/strA/HCM1.222/TetA/strB/STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*
8460	AMP,S,CTX,TE	*BlaTEM-1/SeAg_B4524/strA/strB/blaCTX-M-55/bla CTX-M-57/qnrS/aph6/VCD_003731/blatem precursor/HCM1.222/TetA/STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*
8461	AMP,C,CTX,TE	*SeAg_B4524/blaCTX-M-55/bla CTX-M-57/qnrS/TetA/STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*
8462	AMP,S,NA,CTX,TE	*BlaTEM-1/SeAg_B4524/blaCTX-M-55/bla CTX-M-57/qnrS/aph6/VCD_003731/blatem precursor/strA/strB/HCM1.222/strA/TetA/strB/STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/STM0354/STM1619/STM0580*
8463	AMP,S,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/aph6/VCD_003731/blatem precursor/HCM1.222/STM0352.S/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*
8464	AMP,S,TE	*TetR/BlaTEM-1/SeAg_B4524/strA/strB/aph6/VCD_003731/blatem precursor/HCM1.222/TetA/STM0352.S/sdiA/gyrB/outer membrane efflux-like protein/soxS/cation efflux system protein/parE/STM0354/STM1619/STM0580*
8465	AMP,S,TE	*BlaTEM-1/SeAg_B4524/sul3/dfrA12/aadA1/blatem precursor/aadA2/cmlA1/soxS/dfrA12*
8466	AMP,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/aadA2/qacEdelta 1/soxS*

Table 5.3 (continued)

ID	Antimicrobial resistance	
	Phenotype	**Gene**
8467	AMP,SXT,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/aadA2/qacEdelta 1/soxS*
8468	AMP,SXT,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/aadA2/qacEdelta 1/soxS*
8469	AMP,SXT,C,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/cmlA1/aadA2/soxS*
8470	AMP,SXT,C,S	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/cmlA1/aadA2/soxS*
8471	All Susceptible	*TetR/SeAg_B4524/cueR/soxS*
8472	AMP,SXT,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/dfrA12/aadA1/blatem precursor/aadA1/dfrA12/aadA2/qacEdelta 1/soxS*
8473	AMP,SXT,C,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/mph(A)/blatem precursor/aadA/soxS*
8474	AMP,SXT,C,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/cmlA1/aadA1/aadA2/soxS*
8475	AMP,SXT,C,S,TE	*TetR/SeAg_B4524/cueR/soxS*
8476	AMP,SXT,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/strA/strB/aph6/blatem precursor/soxS*
8477	AMP,SXT,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/cmlA1/aadA2/soxS*
8478	AMP,SXT,C,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/dfrA12/aadA1/blatem precursor/cmlA1/aadA2/soxS*
8479	AMP,SXT,C,S,TE	*TetR/SeAg_B4524/cueR/sul3/dfrA12/aadA1/dfrA12/aadA2/qacEdelta 1/soxS*
8480	TE	*TetR/SeAg_B4524/cueR/soxS*
8481	TE	*TetR/SeAg_B4524/cueR/soxS*
8482	TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/aadA2/qacEdelta 1/soxS*
8483	TE	*TetR/SeAg_B4524/cueR/soxS*
8484	AMP,SXT,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/aadA2/qacEdelta 1/soxS*
8485	AMP,SXT,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/aadA2/qacEdelta 1/soxS*
8486	AMP,S,TE,SXT	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/aadA2/qacEdelta 1/soxS*

Table 5.3 (continued)

ID	Antimicrobial resistance	
	Phenotype	**Gene**
8487	All Susceptible	*TetR/SeAg_B4524/cueR/soxS*
8488	AMP,C,S,TE	*BlaTEM-1/SeAg_B4524/blatem precursor/soxS*
8489	AMP,SXT,TE	*BlaTEM-1/SeAg_B4524/blatem precursor/soxS*
8490	AMP,SXT,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/aadA2/qacEdelta 1/soxS*
8491	AMP,SXT,C,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/cmlA1/aadA2/soxS*
8492	AMP,SXT,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/aadA2/qacEdelta 1/soxS*
8493	AMP,SXT,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/aadA2/qacEdelta 1/soxS*
8494	AMP,SXT,C,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/cmlA1/aadA2/soxS*
8495	AMP,SXT,S,TE	*BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/aadA2/qacEdelta 1/soxS*
8496	AMP,SXT,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/dfrA12/aadA1/blatem precursor/qacEdelta 1/soxS*
8497	AMP,SXT,S,TE	*BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/aadA2/qacEdelta 1/soxS*
8498	AMP,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/dfrA12/blatem precursor/aadA2/qacEdelta 1/soxS*
8500	AMP,SXT,C,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/qnrS/sul3/dfrA12/aadA1/dihydropteroate synthase/blatem precursor/ECL_03814/aadA2/sulII/qacEdelta 1/soxS*
8501	AMP,SXT,C,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/dfrA12/aadA1/blatem precursor/cmlA1 /soxS*
8502	All Susceptible	*SeAg_B4524/soxS*
8503	All Susceptible	*SeAg_B4524/soxS*
8504	All Susceptible	*SeAg_B4524/soxS*
8505	AMP,C,S	*BlaTEM-1/SeAg_B4524/TEM-33/sul3/blatem precursor/aadA/cmlA1/aadA2/soxS*
8506	All Susceptible	*BlaTEM-1/outer membrane efflux-like protein*

Table 5.3 (continued)

ID	Antimicrobial resistance	
	Phenotype	**Gene**
8507	All Susceptible	*BlaTEM-1/outer membrane efflux-like protein*
8508	AMP,SXT,C,S,TE	*BlaTEM-1/SeAg_B4524/sul3/dfrA12/blatem precursor/cmlA1/soxS*
8509	AMP,SXT,C,S,TE	*SeAg_B4524/sul3/dfrA12/aadA2/cmlA1/aadA1/soxS*
8510	AMP,SXT,C,S,TE	*BlaTEM-1/SeAg_B4524/strA/strB/sul3/dfrA12/aph6/aadA1/blatem precursor/aadA2/cmlA1/soxS*
8511	AMP,SXT,C,S,TE	*BlaTEM-1/SeAg_B4524/strA/strB/sul3/dfrA12/aph6/aadA1/blatem precursor/aadA2/cmlA1/soxS*
8512	AMP,C,S,TE,SXT	*BlaTEM-1/SeAg_B4524/sul3/dfrA12/aadA1/blatem precursor/aadA2/cmlA1/soxS*
8513	All Susceptible	*SeAg_B4524/soxS*
8514	AMP,SXT,C,NA,TE	*BlaTEM-1/qnrS/sul3/dfrA12/aadA1/dihydropteroate synthase/blatem precursor/ECL_03814/sulII/cmlA1/aadA2/sul2*
8515	AMP,SXT,C,NA,TE	*BlaTEM-1/qnrS/sul3/dfrA12/aadA1/dihydropteroate synthase/blatem precursor/ECL_03814/sulII/cmlA1/aadA2*
8516	AMP,C,NA,TE,SXT	*BlaTEM-1/qnrS/sul3/dfrA12/aadA1/dihydropteroate synthase/blatem precursor/ECL_03814/sulII/aadA2*
8517	AMP,C,NA,S,TE,SXT	*BlaTEM-1/qnrS/sul3/dfrA12/aadA1/dihydropteroate synthase/blatem precursor/ECL_03814/sulII/cmlA1/aadA2*
8518	AMP,C,NA,TE,SXT	*BlaTEM-1/qnrS/sul3/dfrA12/aadA1/dihydropteroate synthase/blatem precursor/ECL_03814/sulII/cmlA1/aadA2*
8519	AMP,TE	*BlaTEM-1/SeAg_B4524/ blatem precursor/soxS*
8520	AMP,TE	*BlaTEM-1/SeAg_B4524/ blatem precursor/soxS*
8521	AMP,TE	*BlaTEM-1/SeAg_B4524/blatem precursor/soxS*
8522	AMP,S,TE	*BlaTEM-1/SeAg_B4524/strA/strB/aph6/blatem precursor/soxS*
8523	AMP,S,TE	*BlaTEM-1/blaCTX-M-55/bla CTX-M-57/qnrS/blatem precursor*
8524	AMP,S	*BlaTEM-1/SeAg_B4524/strA/strB/aph6/blatem precursor/soxS*
8525	AMP,S	*BlaTEM-1/SeAg_B4524/strA/aph6/blatem precursor/strB/soxS*
8526	AMP,S	*BlaTEM-1/SeAg_B4524/strA/strB/aph6/blatem precursor/soxS*

Table 5.3 (continued)

ID	Antimicrobial resistance	
	Phenotype	**Gene**
8527	AMP,S,TE	*BlaTEM-1/SeAg_B4524/strA/TEM-33/VCD_003731/dihydropteroate synthase/blatem precursor/ECL_03814/sulII/soxS*
8528	AMP,TE	*SeAg_B4524/soxS*
8529	All Susceptible	*SeAg_B4524/soxS*
8530	All Susceptible	*SeAg_B4524/soxS*
8531	AMP,TE	*BlaTEM-1/SeAg_B4524/blatem precursor/soxS*
8872	S	
8873	ALL SUSCEPTIBLE	*SeAg_B4524/soxS*
8874	S,TE	*strA/strB/aph6/VCD_003731/dihydropteroate synthase/ECL_03814/strB/sulII*
8875	AMP,SXT,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/aadA1/blatem precursor/aadA2/qacEdelta 1/soxS*
8876	AMP,SXT,S,TE	*TetR/BlaTEM-1/SeAg_B4524/cueR/sul3/aadA1/blatem precursor/aadA2/qacEdelta 1/soxS*
8877	AMP,C,S,TE	*TetR/BlaTEM-1/sul3/blatem precursor/aacC4/cmlA1/aadA2*
8878	ALL SUSCEPTIBLE	*TetR/SeAg_B4524/cueR/parE/soxS*
8879	S	*STM0580 encodes regulatory protein*
8880	S	*TetR/BlaTEM1/SeAg_B4524/cueR/strA/sul1/strB/dfrA12/aph6/mphA/ACICU_00228/ABAYE3616/dihydropteroate synthase/blatem precursor/ECL_03814/qacEdelta 1/sulI/strB/strA/soxS/hypothetical protein. ORF4*
8881	AMP,AUG,C,S,TE	*TetR/SeAg_B4524/cueR/strA/sul1/strB/aph6/blaCMY2/ACICU_00228/sulI/ABAYE3616/aadA1/VCD_003731/aac3-VI/qacEdelta 1/soxS/hypothetical protein. ORF4*
8882	ALL SUSCEPTIBLE	*TetR/SeAg_B4524/cueR/soxS*
8883	NA	

Table 5.3 (continued)

ID	Antimicrobial resistance	
	Phenotype	**Gene**
8953	AMP,SXT,C,S,TE	*SeAg_B0619/BlaTEM-1/tetd/cueR/strA/sul1/strB/dfrA12/aph6/mphA/blaTEM-1/aadA/qacEdelta1/soxS/hypothetical protein. ORF4*
8954	AMP,SXT,C,S,TE	*SeAg_B0619 /BlaTEM-1/tetd/cueR/sul3/dfrA12/aadA1/blaTEM-1/cmlA1/soxS/mef(B)*
8955	AMP,SXT,S,TE	*SeAg_B0619/BlaTEM-1/tetd/cueR/sul3/dfrA12/aadA1/qacEdelta 1/soxS*
8956	S,NA	
8957	C,S,TE	*SeAg_B0619/sul3/aacC4/aadA/cmlA1*
8958	AMP,SXT,S,TE	*SeAg_B0619/BlaTEM-1/tetd/cueR/sul3/dfrA12/aadA1/qacEdelta1/soxS*

SeAg_B4524 confer transposon tn10 tetd protein

soxS encodes DNA-binding transcriptional regulator SoxS, confers resistance to redox-cycling compounds and antibiotics.

TetR, TetA confer Tetracycline resistance

blaTEM-1, blaCTX-M-14, blaCTX-M-18, blaCTX-M-55, bla CTX-M-57, blatem precursor confer extended-spectrum β-lactamases (ESBLs)

cueR confers cu(i)-responsive transcriptional regulator

sul1,2,3 confer sulfonamide-resistant

dfrA12 confers resistance to trimethoprim

aadA1, aadA2 confer resistance to aminoglycoside

qacEdelta 1 confers resistance to quaternary ammonium compounds (QACs)

strA,strB encodes streptomycin-inactivating enzymes, are confer streptomycin resistance

VCD_003731 encodes aminoglycoside 3'-phosphotransferase, confer aminoglycoside resistance

aph4, aph6 confers aminoglycoside resistance

aacC4 confers resistance to apramycin

STM0352.S encodes cation efflux system protein

sdiA encodes ftsQAZ transcriptional regulator** read more

gyrB confers Nalidixic acid-resistant mutation

parE confers fluoroquinolone resistance

STM0354 encodes transcriptional regulator

STM1619 encodes aminoglycoside N(6')-acetyltransferase

STM0580 encodes regulatory protein

HCM1.222 encodes streptomycin phosphotransferase

cmlA1 encodes chloramphenicol transporter

ECL_03814 encodes dihydropteroate synthase

ACICU_00228 encodes dihydropteroate synthase

ABAYE3616 encodes dihydropteroate synthase

qnrS confers plasmid-mediated quinolone resistance gene

mph(A) confer Macrolides resistance

FosA4 encodes Fosfomycin resistance glutathione S-transferase

aac3-VI confers gentamicin resistance

Hypothetical protein. ORF4 similar to uncultured bacterium pB8 dihydropteroate synthase

mef(B) encodes macrolide efflux pump

Table 5.4 Odds ratio and P-value of the antimicrobial resistance gene and phenotype.

Antimicrobial resistance agent	(+G)(+P)	(-G)(+P)	(+G)(-P)	(-G)(-P)	Odds rations	Significance level	Sensitivity	Specificity
Chloramphenicol	23	14	4	83	34.1	$P < 0.0001$	62.2	95.4
Streptomycin	76	7	19	22	12.6	$P < 0.0001$	91.6	53.7
Ampicillin	90	5	6	23	69.0	$P < 0.0001$	94.7	79.3
Cefotaxime	11	0	85	28	N/A	N/A	100.0	24.8
Ciprofloxacin	0	0	31	87	N/A	N/A	N/A	73.7
Norfloxacin	0	0	31	87	N/A	N/A	N/A	73.7
Nalidixic acid	6	0	31	87	N/A	N/A	100.0	73.7
Tetracycline	85	8	23	8	3.7	$P = 0.0180$	91.4	25.8
Trimethoprim/sulfamethoxazole	46	1	17	60	162.4	$P < 0.0001$	97.9	77.9

Table 5.5 Prevalence of antimicrobial resistance genes in swine production chain, Thailand

Antimicrobial/resistance mechanism genes	Farm (40 isolates)		SLH (54 isolates)		Market (32 isolates)		Total (126 isolates)	
	%	(No.)	%	(No.)	%	(No.)	%	(No.)
Chloramphenicol	20	(8)	22.2	(12)	15.6	(5)	19.8	(25)
Aminoglycoside	82.5	(33)	61.1	(33)	68.8	(22)	69.8	(88)
Apramycin	0	(0)	0.0	(0)	9.4	(3)	2.4	(3)
Cation efflux system protein	47.5	(19)	13.0	(7)	9.4	(3)	23.0	(29)
Cu(i)-responsive transcriptional regulator	30	12)	38.9	(21)	43.8	(14)	37.3	(47)
ESBL	82.5	(33)	75.9	(41)	59.4	(19)	73.8	(93)
Fluoroquinolone	50	(20)	20.4	(11)	15.6	(5)	28.6	(36)
Fosfomycin	2.5	(1)	1.9	(1)	0.0	(0)	1.6	(2)
ftsQAZ transcriptional regulator	40	(16)	11.1	(6)	9.4	(3)	19.8	(25)
Gentamicin	5	(2)	0.0	(0)	3.1	(1)	2.4	(3)
Macrolides	7.5	(3)	5.6	(3)	6.3	(2)	6.3	(8)
Nalidixic acid	47.5	(19)	13.0	(7)	9.4	(3)	23.0	(29)
Outer membrane efflux-like protein	47.5	(19)	16.7	(9)	9.4	(3)	24.6	(31)
Quaternary ammonium compounds (QACs)	20	(8)	22.2	(12)	34.4	(11)	24.6	(31)
Redox-cycling compounds and antibiotics	90	(36)	88.9	(48)	65.6	(21)	83.3	(105)
Regulatory protein	47.5	(19)	13.0	(7)	12.5	(4)	23.8	(30)

Table 5.5 (continued)

Antimicrobial/resistance mechanism genes	Farm (40 isolates)		SLH (54 isolates)		Market (32 isolates)		Total (126 isolates)	
	%	(No.)	%	(No.)	%	(No.)	%	(No.)
Sulfonamide	45	(18)	40.7	(22)	53.1	(17)	45.2	(57)
Tetracycline	87.5	(35)	88.9	(48)	78.1	(25)	85.7	(108)
Transcriptional regulator	47.5	(19)	13.0	(7)	9.4	(3)	23.0	(29)
Trimethoprim	37.5	(15)	44.4	(24)	28.1	(9)	38.1	(48)

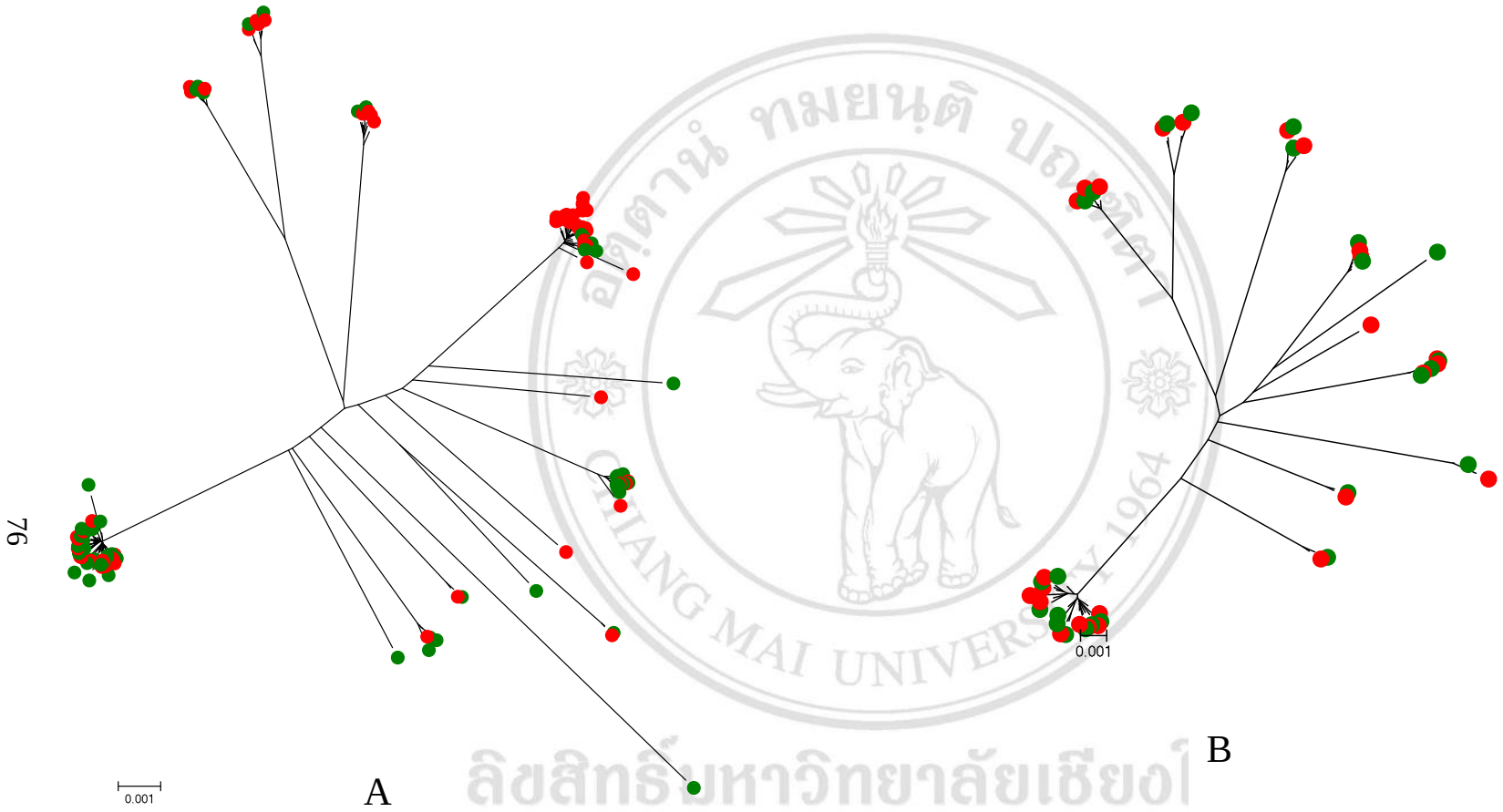

Figure 5.4 (A) Neighbor-joining tree of all isolates base on biofilm formation. High biofilm formation is red. Low biofilm formation is green. (B) Tree of choosing for GWAS study

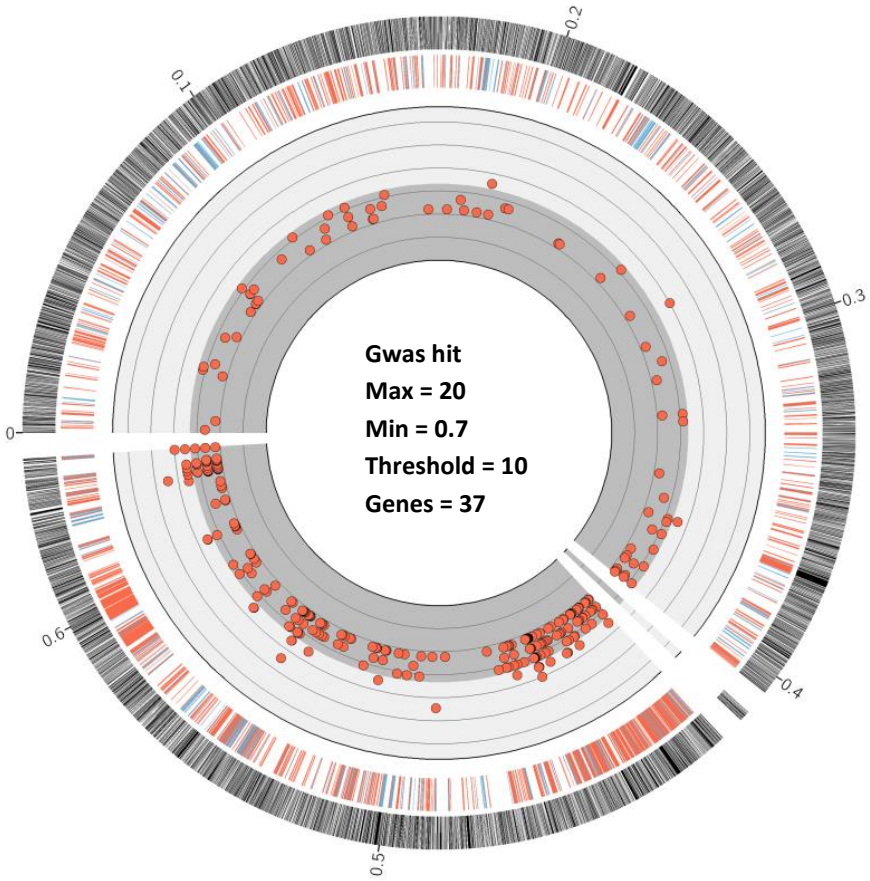

Figure 5.5 Gwas hit associated with survival of *Salmonella spp*. in swine production chain

Locus	Description	Score
id8525_4842	[NiFe]_hydrogenase_nickel_incorporation_protein_HybF	20
id8432_1639	Putative_heat_shock_protein_YegD	20
id8426_3158	Mobile_element_protein	16
id8880_0530	Polymyxin_resistance_protein_PmrJ,_predicted_deacetylase	16
id8426_3160	Tn7-like_transposition_protein_D	16
id8431_2428	Zinc_uptake_regulation_protein_ZUR	16
id8880_1073	Ferric_enterobactin_uptake_protein_FepE	14
id8432_3713	Fimbrial_protein_YadN-like	14
id8432_5104	Macrolide_export_ATP-binding/permease_protein_MacB_(EC_3.6.3.-)	14
id8956_2266	Potassium_efflux_system_KefA_protein_/_Small-conductance_mechanosensitive_channel	14
id8956_0316	Sigma_factor_RpoE_negative_regulatory_protein_RseA	14
id8431_2419	SOS-response_repressor_and_protease_LexA_(EC_3.4.21.88)	14
id8426_4090	Alpha-fimbriae_tip_adhesin	12
id8487_4779	Beta-fimbriae_probable_major_subunit	12
id8436_2003	Cobalt-zinc-cadmium_resistance_protein_CzcA;_Cation_efflux_system_protein_CusA	12
id8426_3084	Fimbriae_usher_protein_StfC	12
id8880_0133	Fimbriae-like_adhesin_SfmA	12
id8956_1508	Integrase	12
id8426_1813	Predicted_N-ribosylNicotinamide_CRP-like_regulator	12
id8880_2177	Transcription_repressor_of_multidrug_efflux_pump_acrAB_operon,_TetR_(AcrR)_family	12
id8880_2547	Zinc_ABC_transporter,_inner_membrane_permease_protein_ZnuB	12
id8487_4778	Beta-fimbriae_probable_major_subunit	10
id8498_0188	Fimbrial_protein_YadM-like	10
id8526_1081	Flagellar_biosynthesis_protein_FliL	10
id1_Salm_chr_1857	Flagellar_biosynthesis_protein_FliP	10
id8438_4214	Flagellar_hook-basal_body_complex_protein_FliE	10
id8426_2373	Integrase	10
id1_Salm_chr_3502	Lipopolysaccharide_core_biosynthesis_protein_RfaY	10
id8880_2753	Mobile_element_protein	10
id8431_4314	Molybdenum_cofactor_biosynthesis_protein_MoaA	10
id8510_0742	Multidrug-efflux_transporter,_major_facilitator_superfamily_(MFS)_(TC_2.A.1)	10
id8487_3172	NAD(P)H-flavin_reductase_(EC_1.5.1.29)_(EC_1.16.1.3)	10
id8880_3142	Ni,Fe-hydrogenase_I_cytochrome_b_subunit	10
id8489_4305	Predicted_outer_membrane_lipoprotein_YfeY	10
id8531_1400	Universal_stress_protein_G	10
id8447_0931	Copper_resistance_protein_D	10
id8426_2370	Integrase	10

Figure 5.5 (continued)

5.5 Discussion

Thai *Salmonella* isolates were not quite much differed from global collection. However, *S.* Rissen was major serotypes in this study which have very rare data in NCBI. *S.* Rissen might be the major serotypes in this region [103]. The nucleotide difference tree (ND tree) was used for epidemiological study of *Salmonella* in this study, which based on nucleotide difference between a pair of read mapped reference genomes. ND trees were a superior method for clustering outbreak related isolates of *Salmonella* spp. [34]. The contamination of *Salmonella* spp. along swine food production might be found in this study. The contamination between farm to slaughterhouse was shown in Id (8517-8515), (8440-8459), (8442-8441), (8529-8530), (8474-8481), (8501-8475) and (8485-8467). *Salmonella* form pig farms were transmit and contaminated inside slaughtering process. *Salmonella* in same genetic can be found on carcrass, mesenteric lymph node, pig's skin, knife and hand's worker (data not show). The transportation and cleaning step before letting pigs come in slaughterhouse should be improved to decrease the opportunity of contamination in slaughtering process.

The contamination of *Salmonella* between farm to farm (same farm) was also found in this study in id (8456-8457), (8460-8461), (8443-8447) and (8472-8486). Interestingly, most of them (4/5 pairs) were collected samples in different date (data not show). This show that there was the persistence strains in the farm. The reason might be from the cleaning program is not quite good, resistance to antiseptic or biofilm formation.

Furthermore, the contamination along slaughtering process (same slaughterhouse) was shown in this study in Id (8510-8511), (8520-8521), (8477-8478), (8491-8494), (8466-8490) and (8479-8493).All of them were collected in the same date. The critical processes of contamination from these results were recovered from various slaughtering process steps such as lairage, splitter, evisceration, washing or chilling. The lacks in that in routine swine production practices also promote the colonization and spread of *Salmonella* to pork via pig's skin, contaminated carcasses, slaughtering equipment or worker's hands at any of the slaughtering-processes [11, 14, 126].

Contamination from slaughterhouse to market was also found in this study in Id (8480-8875). Critical point in farms and slaughterhouse should be control to reduce the risk of contamination of *Salmonella* to market places [127]. In fact, market place should not be found any *Salmonella* contamination because they are the place that contributed pork to community. Furthermore, they would be carried antimicrobial resistance *Salmonella* as well. Thus, undercook meat and good hygiene practice should be performed.

The relationship between resistance phenotype and associated resistance gene were explored by CARD with Gene-by-gene approach by Bigsdb genome comparator tool. It is good, convenient and fastest tool for analyses antimicrobial resistance genes. From table 5.5, the relationship of Chloramphenicol, Streptomycin, Ampicillin, Tetracycline and Sulfamethoxazole-Trimethoprim resistance genes and their phenotypes were detected ($p<0.05$). The chance of isolates harboured resistance gene will be more express their phenotype than other isolate were up to 34.1, 12.6, 69, 3.7 and 162 times, respectively. The sensitivity and specificity calculated from the outcomes were provided good concordance between resistance phenotype and associated resistance genes. The presence or absence of resistance genes could be predicted the phenotype in most occasions. However, some occasions between in phenotype expression and genotype were not match well. In chloramphenicol, some non-gene harbouring isolates with positive phenotypic finding were observed. That might be the selection pressure, mutation and survivorship was taken place on this situation that act on phenotype. Moreover, it might be the antimicrobial resistance CARD does not cover all resistance genes. In contrast, tetracycline, some isolates carried resistance gene without showing ability of resistance on phenotype were identified. The resistance gene might be off of function due to environmental interaction [128].

Interestingly, in this study found Extended-spectrum beta-lactamases (ESBLs) which is enzymes recognizing a cause of resistance to 1^{st}-4^{th} generation of cephalosporin and aztreonam [53]. Furthermore, multidrug resistance genes were found in this study. There is the very high prevalence of tetracycline and aminoglycoside resistance genes. Both of them are used as growth promotor in livestock in Thailand. They both are not able to be the drug of choice for salmonellosis in the future. Infection of this strain will

be progress to more serious which can be life threatening. Multidrug resistance makes these infections more difficult to treat. Furthermore, many strains harbour antiseptic resistance genes including outer membrane efflux-like protein, cation efflux system protein and quaternary ammonium compound resistance gene (qecRdelta1). This might the one reason why *Salmonella* spp. is very difficult to eradicate on swine production chain and *Salmonella* spp. is very harmful for human, animal and environment as well. Additionally, from the results of the study can be predict the ability of antimicrobial resistance of *Salmonella* spp. in the future. Using of antibiotic and antiseptic agent should be under the control and direction of a veterinarian in livestock and hospital by doctor and pharmacist. The right dose, right time, best route and choosing an appropriate of antimicrobial agents should be concern. That can increase efficiency of treatment of infection. Education and training on food handling and food consumption are also important ways to help prevent foodborne illnesses and spreading of resistance gene to communities [35].

There are 37 genes *Salmonella spp*. associated with survival of this pathogen. There were about metal uptake, antimicrobial resistance, antiseptic resistance, mobile genetic elements (integrons, transposon) stress responses induced within biofilms (Heat shock protein, sigma factor, SOS response) [129] finding gene, genes involved in biofilm formation (motility gene, CRP:Repress biofilm formation, LPS) [130]. Beside many genes which associated with survival of *Salmonella* spp. was found in this study; Curri, multidrug resistance. However, they are not high score of association. *Salmonella* can survive along swine production with many kinds of processes. This study can be confirmed why this pathogen are persisted and very hard to eradicate in swine production chain. Good management of antiseptic and antimicrobial usage is very important to concern.

Some of *Salmonella* strains were test to compare the ability of distinguish genetic diversity. The Simpson's index of diversity of PFGE and WGS were 0.919 and 0.993, respectively, indicating the high discriminatory power of these two techniques. However, WGS had a little bit higher differentiation ability than PFGE for *Salmonella* strains, comparable to the results in the study of [34]. The concordance between WGS

and PFGE was examined by calculating the Adjusted Rand and Wallace coefficients. The Adjusted Rand coefficient was 0.057, which indicated a low congruence between PFGE and WGS (From 7 clusters of PFGE and 8 clusters of WGS were generated. The Wallace coefficient of PFGE to WGS was 0.037 which indicates that if the isolates were recognized as having the same PFGE type, those isolates had a 3.7% chance of being identified as the same WGS results. However, Wallace coefficient of WGS to PFGE was 0.455 indicating that if the isolates were identified as having the same WGS results, those isolates had a 45.5% chance of being identified as the same PFGE [78].

PFGE has been a stand typing for epidemiological approach of *Salmonella*. However, it is unable to distinct very closely related strains because the low rate of genetic variation does not express on electrophoretic fragment [131]. Whole-genome sequencing (WGS) has become a significant and rapidly handy tool for microbial identification, pathogenesis, comparative analyses and outbreak investigation [34, 132]. The whole-genome sequencing also is unbiased detection of other information about the strains that the clinician may not have considered, such as the unexpected presence of antibiotic-resistance gene [120].

5.6 Conclusion

Whole genome sequencing is becoming the rapidly tool for outbreak investigation of *Salmonella* as well as transmission, genetic analysis and microbial identification. Furthermore, this tool is useful for prediction of antimicrobial resistance which help for surveillance the emerging of antimicrobial resistance pathogen. Education of antimicrobial usage should be controlled by veterinarian, doctor and pharmacist. Cleaning programme in swine production should be improved. Having raw meat or non-undercook meat should be avoided.

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