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1 **Core genome sequence analysis to characterize *Salmonella enterica* serovar Rissen ST469**
2 **from a swine production chain**

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7 **Highlights**

8 - *Salmonella* serovar Rissen from the different stages of the pork production chain were
9 genetically related based on cgMLST analysis.
10 - *Salmonella* serovar Rissen can persist and cross/re-contamination can occur in all steps
11 of the pork production chain.
12 - Phenotypic resistance to antimicrobials are accurately predicted with high sensitivity
13 and specificity using WGS data.

14 **Abstract**

15 *Salmonella enterica* subsp. *enterica* serotype Rissen is the predominant serotype found
16 in Thai pork production and can be transmitted to humans through contamination of the food
17 chain. This study was conducted to investigate the genetic relationships between serovar Rissen
18 isolates from all levels of the pork production chain and evaluate the ability of the *in silico*
19 antimicrobial resistance (AMR) genotypes to predict the phenotype of serovar Rissen. A total
20 of 38 serovar Rissen isolates were tested against eight antibiotic agents by a disk diffusion
21 method and the whole genomes of all isolates were sequenced to detect AMR genetic elements
22 using the ResFinder database. A total of 86.84% of the isolates were resistant to tetracycline,
23 followed by ampicillin (78.96%) and sulfonamide-trimethoprim (71.05%). Resistance to more
24 than one antimicrobial agent was observed in 78.95% of the isolates, with the most common
25 pattern showing resistance to ampicillin, chloramphenicol, streptomycin, sulfonamide-
26 trimethoprim, and tetracycline. The results of genotypic AMR indicated that 89.47% of the
27 isolates carried *tet(A)*, 84.22% carried $bla_{TEM\text{-}1B}$, 78.95% carried *sul3*, and 78.95% carried
28 *dfrA12*. The genotypic prediction of phenotypic resistance resulted in a mean sensitivity of
29 97.45% and specificity of 75.48%. Analysis by core genome multilocus sequence typing
30 (cgMLST) demonstrated that the *Salmonella* isolates from various sources and different
31 locations shared many of the same core genome loci. This implies that serovar Rissen has
32 infected every stage of the pork production process and that contamination can occur in every
33 part of the production chain.

34

35 **Key words:** *Salmonella* serovar Rissen; antimicrobial resistance; whole genome sequencing;
36 core genome MLST; pig production

37

Introduction

Salmonella is a genus of gram- negative, rod- shaped bacteria in the family Enterobacteriaceae. *Salmonella enterica* (*S. enterica*) is divided into six subspecies: *enterica, salamae, arizonae, diarizonae, houtenae,* and *indica* (Frasson et al., 2016). *S. enterica* subsp. *enterica* includes more than 2,600 serotypes that have the ability to infect in humans and warm-blooded animals (Velge et al., 2012). This pathogen is one of the most important bacterial diseases in food animals throughout the world. *Salmonella* infection in farm animals is the leading cause of economic losses in the global livestock production industry (Bengtsson and Greko, 2014). In Asian countries, *Salmonella enterica* subsp. *enterica* serovar Rissen (serovar Rissen) is typically associated with the swine production chain that extends from farms to slaughterhouses and retail outlets (Lim et al., 2009; Sinwat et al., 2016; Thai and Yamaguchi, 2012). The occurrence of *Salmonella* infection at the herd level indicates that farms could be the origin of contamination in meat (Alpigiani et al., 2014). Several epidemiological studies have indicated that pork is a source of infection for human salmonellosis (Evangelopoulou et al., 2014). Thus, reduction of *Salmonella* in the pig supply chain is crucial for human health and food security (Toro et al., 2016).

The global development of antimicrobial resistance (AMR) in foodborne pathogens is a particular public health concern, especially in non-typhoidal *Salmonella* species. Multidrug resistance (MDR) in *Salmonella* and other enteric pathogens has occurred on multiple continents and can cross international boundaries (Iwu et al., 2016). The livestock sector is a suspected reservoir of bacteria carrying MDR. The use of antimicrobials in agricultural animals for disease treatment and prevention, as well as secondary use as a growth promoter can promote selection of antimicrobial resistant bacteria (Exner et al., 2017; Magouras et al., 2017).

61 Virulence factors and antimicrobial resistance genes can also be found on plasmids, such as
62 the incompatibility group (Inc) of plasmids, or clustered on *Salmonella* pathogenicity islands
63 (SPIs) (Espinoza et al., 2017; Han et al., 2012; Nieto et al., 2016).

64 Classical typing methods such as phage typing and serotyping are limited to
65 differentiation within the same species. Molecular typing methods, such as pulsed-field gel
66 electrophoresis (PFGE) have been used successfully for *Salmonella* typing and are now
67 considered the gold standard for typing *Salmonella* strains (Salipante et al., 2015). However,
68 even these typing methods cannot discriminate between highly clonal strains (Bekal et al.,
69 2016). At present, whole genome sequencing (WGS) offers a more powerful characterization
70 than PFGE(Ibrahim and Morin, 2018). WGS is very useful in food safety improvement and in
71 establishing preventive control measures for foodborne diseases (Moran-Gilad, 2017). WGS
72 data can also allow re-analysis for detection of antimicrobial resistance genes, virulence
73 factors, and mobile genetic elements (Ronholm et al., 2016).

74 The objective of the present study was to use WGS to describe the genetic relationship
75 among the serovar Rissen isolates obtained at different stages of the swine production chain.
76 In addition, the ability of the AMR genotype to predict the phenotypic characteristics was also
77 assessed.

78

79 **Materials and Methods**

80 **Bacterial strains and molecular typing**

81 All serovar Rissen isolates analyzed in this study were collected as part of previous
82 studies (Patchanee et al., 2016; Tadee et al., 2015). The samples were collected from pig farms
83 (n=12), pig slaughterhouses (n=22), and retail outlets (n=4) around Chiang Mai and Lamphun
84 during 2012–2014. *Salmonella* were cultured according to ISO 6579:2002 Amendment 1:2007,

85 Annex D at Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand.
86 Serotyping and antimicrobial susceptibility testing were performed at the WHO National
87 *Salmonella* and *Shigella* Center, Department of Medical Science, Nonthaburi, Thailand. A
88 summary of the *Salmonella* strains used in this study is presented in Table 1.

89

90 **Whole Genome Sequencing**

91 DNA was extracted from all samples with a QIAamp DNA Mini Kit (Qiagen, Crawley,
92 UK). The library was prepared according to the manufacturer's instructions using the Nextera
93 XT DNA Library Preparation Kit (Illumina, Cambridge UK). The *Salmonella* genomes were
94 sequenced using Illumina MiSeq 300bp paired-end sequencing technology (v3 run kit;
95 Illumina, Cambridge UK). The genomes of serovar Rissen were assembled *de novo* with
96 SPAdes software (version 3.8.0, using the *careful* command)(Bankevich et al., 2012). All
97 genomes used in this study were archived on the BIGSdb web-based database platform (REFS):
98 https://sheppardlab.com/resources/ using *S.* Typhimurium LT2 (accession number
99 NC_003197) to identify reference loci. Sequenced shorts reads have been depositted with
100 NCBI associated with the BioProject# PRJNA540675.

101

102 **Identification of antimicrobial resistance genes, *Salmonella* pathogenicity islands, MLST**
103 **sequence type, and plasmid profiling**

104 The FASTA files of 38 *Salmonella* Rissen strains were investigated for antimicrobial
105 resistance genes using the ResFinder 3. 0 database available at
106 https: / / cge. cbs. dtu. dk/ services/ ResFinder/ (Zankari et al., 2012). The investigated
107 antimicrobial resistance genes included aminoglycoside (*aad*A1, *aad*A2, *aph*3, *aph*6, and
108 *str*A), beta-lactam (bla_{TEM-1B}), quinolone (*qnr*S1), macrolide (*mph*(A) and *mef*(B)), phenicol

109 (*cml*A1, *cml*, and *flo*R), sulfonamide (*sul*1, *sul*2, and *sul*3), tetracycline (*tet*(A) and *tet*(M)), and
110 trimethoprim (*dfr*A12) resistance genes. The *Salmonella* pathogenicity islands (SPI), MLST
111 sequence type, and plasmid were examined by SPIFinder 1.0
112 (https://cge.cbs.dtu.dk/services/SPIFinder/) (Kozyreva et al., 2016), MLST 2.0
113 (https://cge.cbs.dtu.dk/services/MLST/), and PlasmidFinder 2.0
114 (https://cge.cbs.dtu.dk/services/PlasmidFinder/) (Carattoli et al., 2014; Larsen et al., 2012).
115
116 **The correlation between AMR genotype and phenotype**
117 The sensitivity of AMR genotype prediction was calculated by the number of resistance
118 phenotypes divided by the total number of isolates exhibiting AMR phenotypes. Specificity
119 was also calculated by dividing the number of the susceptible genotypes by the total number
120 of isolates with susceptible phenotypes. The receiver operating characteristic (ROC) curves
121 were analysed to determine antimicrobial resistant phenotype of corresponding genes. The area
122 under the ROC curve (AUC) was calculated to evaluate the accuracy of the prediction.
123
124 **Analysis by core genome multilocus sequence typing (cgMLST)**
125 The cgMLST analysis was conducted using BioNumerics software version 7.6.3
126 (Applied Maths, Sint-Martens-Latem, Belgium). The wgMLST schema in the software
127 consists of a total of 15,874 loci from 199 of publicy available *Salmonella enterica* reference
128 genomes. The cgMLST analysis was restricted to loci with ≥ 80% homology in ≥ 95% of the
129 isolates (2,516 loci). The minimum spanning tree (MST) was generated using the algorithm for
130 clustering categorical data.
131
132 **Results**

133 **Distribution of MLST, AMR genes, AMR phenotypes, plasmid replicons, and SPIs**

134 A total of 38 serovar Rissen isolates belonged to sequence type (ST) 469 (Table 1), 135 which was classified by seven housekeeping genes: *aro*C 92, *dna*N 107, *hem*D 79, *his*D 156, 136 *pur*E 64, *suc*A 151, and *thr*A 87.

137 The antimicrobial resistance genes and phenotypes of eight antibiotic groups are 138 summarized in table 2. The most common genes were tetracycline resistance genes (*tet(A)*, 139 89.47%), followed by beta-lactam resistance genes (bla_{TEM-1B}, 84.22%) and sulfonamide-140 trimethoprim genes (*sul*3, 78.95% and *dfr*A12, 78.95%). From the results of AMR phenotype, 141 nearly 87% of samples were resistance to TE (Table 2). Multi-drug resistance (MDR) was 142 found in nearly 80% (30/38) of the isolates, while approximately 15% (6/38) of the isolates 143 showed resistance to one antimicrobial agent (TE) and about 5% (2/38) were susceptible to all 144 eight antimicrobial agents. The most common MDR patterns were AMP, C, S, SXT, and TE 145 (31.58%), followed by AMP, S, SXT, and TE (23.68%) and AMP, SXT, and TE (7.89%) 146 (Table 3).

147 Four Incompatibility group (Inc) plasmid replicons were observed within all of the 148 serovar Rissen. The three most commonly found were IncFIB(K) (18.42%), IncFIA(HI1) 149 (15.79%), and IncFIIS (13.16%) (Table 3). All the serovar Rissen isolates examined possessed 150 SPI-3 and SPI-12 (Table 3), whereas 15.79% and 42.11% of the isolates carried SPI-1 and 151 SPI-2, respectively. In this study, both SPI-1 and SPI-2 were found in the R03 isolate, while 152 SPI-4 was present only in the R37 isolate.

153

154 **Genotype predictions of the AMR phenotype**

155 The data for the AMR genotypes and phenotypes in table 2 were used to evaluate the
156 effectiveness of genotypic markers to predict a resistant phenotype. The antimicrobials in the
157 quinolone group were not included for evaluation because no isolates were resistant to CIP,
158 NA, and NOR. The results for the genotypic prediction of phenotypic resistance of AMP, S,
159 C, SXT, and TE are shown in table 4. The mean sensitivity and specificity for genotypic
160 prediction of phenotypic resistance were 97.45% and 75.48%, respectively (Table 4).
161 Genotypic prediction of phenotypic resistance to AMP, S, and SXT had a sensitivity of 100%,
162 followed by C (93.33%) and TE (93.94%). The specificity of the prediction of five antimicrobial
163 agents was more than 70% but TE had the highest specificity (80.00%) (Table 4). The receiver
164 operating characteristic (ROC) curve, used to evaluate the accuracy of the prediction, showed
165 an area under the ROC curve that ranged from 0.85–0.95 and an average accuracy of 90.52%
166 (Table 4).
167

168 **Core genome and whole genome MLST analysis**

169 The cgMLST scheme was analysed by 2,516 core loci shared within all *Salmonella*
170 isolates (Fig. 1). The minimum spanning tree (MST) divided the serovar Rissen isolates into
171 five clusters (yellow, pink, gray, purple, and brown) and five single isolates. The major cluster
172 (purple) contained 19 isolates from different origins: pig slaughterhouses (R17-R19, R25-R26,
173 R31-R34, and R36-R37), pig farms (R09-13), and pork from fresh markets (R01-R03). All
174 isolates in the major cluster had been sampled during 2012 to 2014, and they shared the same
175 core genome. Loci with greater than 80% homology in over 95% of our Salmonella population
176 were included in our cgMLST scheme (Vincent et al., 2018). This conservative approach
177 resulted in 2,516 loci shared in our 38 serovar Rissen genomes. The close genetic relationship

178 between serovar Rissen isolates suggests that *Salmonella* serovar Rissen is highly clonal and
179 may persist throughout the pork production process and contaminate farms and retail meat.

180 The pink and yellow clusters comprising *Salmonella* isolates from the same location
181 and collected on the same day had identical cgMLST profiles (Fig 1). Four isolates (R20, R21,
182 R22, and R23) in the pink cluster came from different sources in the splitting step from
183 slaughterhouse01 on May 19th, 2013 (Table 1) and two isolates (R07 and R08) in the yellow
184 cluster were sampled from the boots of workers at farm02 on Jul 03rd, 2012 (Table 1). These
185 results may indicate that *Salmonella* is spreading between the pig farm and slaughterhouse.
186 However, it may be possible to differentiate these isolates using a Rissen-specific cgMLST
187 scheme or by comparing SNPs (REFS).

188

189 **Discussion**

190 Alarming levels of antimicrobial resistance were identified at each stage of the pork
191 production process. High levels of resistance were detected against tetracycline (86.84%),
192 ampicillin (78.96%), and trimethoprim-sulfamethoxazole (71.05%) and almost 80% of the
193 samples showed MDR (resistance to at least two antimicrobial agents). In the northeastern part
194 of Thailand and Laos, resistance to sulfonamides (98.30%), trimethoprim (49.50%), ampicillin
195 (91.00%), and tetracycline (92.50%) was reported at high frequency in pig production (Sinwat
196 et al., 2016). MDR was also observed in livestock production on the Asian continent, including
197 in Laos (98.2%), China (73.2%), and Taiwan (96%) (Kuo et al., 2014; Sinwat et al., 2016;
198 Zhang et al., 2018). The high prevalence of MDR *Salmonella* in Thailand and Asian swine
199 production is a serious public health risk in this area.

200 Tetracycline resistance genes (*tetA*) were the most frequently detected AMR genes in
201 this study, followed by beta-lactam (bla_{TEM-1B}) and sulfonamide-trimethoprim (*sul3* and

202 *dfrA12*) resistance genes and genotypic markers of resistance were well correlated with the
203 phenotypic resistance profiles. For every antimicrobial group, the number of isolates that
204 carried putative resistance genes was higher than the number of resistant phenotypes, in
205 agreement with several studies that have indicated the existence of silent resistance genes in
206 bacteria (Adesiji et al., 2014; Deekshit et al., 2012). Furthermore, the antimicrobial resistance
207 genes may be located in common genetic elements, associated with other advantageous genes.
208 Thus, resistance genes can be maintained in the genome as consequence of co-selection
209 (Aarestrup, 2005; Srisanga et al., 2017). The transfer of silent antimicrobial resistance genes to
210 other bacteria is possible and can be activated under antibiotic selection pressure (Davis et al.,
211 2011; Zhang et al., 2016).

212 The use of the quinolone antimicrobial group is widespread in veterinary practice.
213 Fortunately, all the 38 serovar Rissen in this study were susceptible to all quinolone groups
214 (ciprofloxacin, nalidixic acid, and norfloxacin). However, the *qnrS1* gene (a quinolone
215 resistance gene) was detected in two samples that were susceptible to all quinolone agents. The
216 *qnrS1* gene commonly appears in plasmid-mediated quinolone resistance (PMQR) in
217 *Salmonella* spp. The *qnrS1* gene of the bacteria in the Enterobacteriaceae family is often found
218 located on the incompatibility groups of the plasmid (Inc), such as IncN and IncX (Carattoli,
219 2013). In this study, we find serovar Rissen isolates with the *qnrS1* gene carried on the IncX1
220 plasmid.

221 The *Salmonella* pathogenicity islands (SPIs) are numerous gene clusters located in the
222 chromosome of *Salmonella* spp. At present, 23 SPIs have been identified but the roles of some
223 SPIs are not clearly understood (Nieto et al., 2016). In our study, SPI-3 and SPI-12 were
224 present in 100% of the serovar Rissen isolates. SPI-3 encodes the *cigR*, *fdL*, *marT*, *mgtB*, and

225 *mgtC* genes. The *mgtB* and *mgtC* genes are related to exposure to tetracycline or
226 chloramphenicol and were found in high frequency in the resistant phenotype, at 86.84% and
227 39.48% for TE and C, respectively. SPI-3 was also required for *Salmonella* survival within
228 macrophages and for growth in low-Mg^{2+} conditions while SPI-12 contributed to bacterial
229 survival in the host (Gerlach and Hensel, 2007; Holman et al., 2018; Tomljenovic-Berube et
230 al., 2013). However, SPI-1 and SPI-2, which are the most important SPIs in *S. enterica,* were
231 found in six and sixteen isolates, respectively. Encoding the type III secretion system (T3SS)
232 is the main function of both SPI-1 and SPI-2, which are required for invasion of intestinal
233 epithelial cells and are essential for *Salmonella* intracellular survival and replication. In the
234 current study, an R03 isolate carried both SPI-1 and SPI-2. *Salmonella* isolates that carried
235 just SPI-1 or SPI-2 were less virulent than strains that had both SPI-1 and SPI-2 (Grant et al.,
236 2012; Nieto et al., 2016). So, carrying SPI-1 and SPI-2 at lower levels within serovar Rissen
237 make this serovar is not very virulent strain.

238 Our study confirmed the effectiveness of predicting phenotypic resistance using
239 genotype data from WGS. *In silico* AMR gene predictions were highly correlated with
240 phenotype characteristics (Table 4). The high sensitivity and specificity of the five
241 antimicrobial groups indicated that WGS data could be used to evaluate the AMR phenotype
242 in *Salmonella*. The ability to predict the phenotype of AMR from the genotype has previously
243 been investigated in various species of bacteria such as *Staphylococcus aureus, Campylobacter*
244 spp., and *Mycobacterium tuberculosis* (Bradley et al., 2015; McDermott et al., 2016; Zhao et
245 al., 2016). In addition, AMR prediction from genotype within Salmonella has been reported in
246 many serovar such as Typhimurium, Newport, and Dublin (Carroll et al., 2017; McDermott et
247 al., 2016). Our findings therefore support the use of WGS as an alternative tool for prognosis

248 of AMR profiles and as a rapid monitoring method for AMR outbreaks, because it is faster
249 than the classical phenotypic AMR testing.

250 All 38 serovar Rissen isolates belonged to ST469 based on their MLST classification
251 (seven housekeeping genes). This result showed that the classical MLST cannot distinguish the
252 *Salmonella* strains in this study, so the core-genome (cg) MLST was used to discriminate the
253 *Salmonella* strains. The cgMLST identification of serovar Rissen from different origins of the
254 swine production chain showed close relationships among some strains (Fig. 1) and yet higher
255 resolution phylogenetic methods may be required to differentiate isolates. Despite isolates
256 being sampled from different locations and time periods, they shared identical cgMLST
257 profiles. The sampling period in this group was interesting as the isolates from farms,
258 slaughterhouses, and markets were collected from May–June, 2012, May–June, 2013, and
259 September–October, 2014, respectively. Given the highly clonal popualtion structure of
260 serovar Rissen, it is unclear if they descended from the same origin.

261 The persistence of serovar Rissen in the pig production chain was observed in Chiang
262 Mai and Lamphun provinces. The cgMLST analysis indicated that the *Salmonella* isolates in
263 the grey, purple and brown clusters were from different years and various origins, but they had
264 a similar core genome (Fig 1), implying a shared ancestor and persistence on the pig farms
265 and every step of the slaughtering process, contaminating slaughterhouses and retail pork
266 produce sold in the fresh markets. *Salmonella* contamination was detected at multiple sites,
267 including pig feces; the workers' hands and boots; the equipment, such as knives used in the
268 slaughtering process; and the environment (e.g., floors, cages, etc.). Cross contamination from
269 one item to another and/or one area to another location is likely by direct contact and reflects
270 the importance of strict monitoring of cleaning and sanitation in the pig production process
271 because *Salmonella* can survive in the environment without infecting a host for more than one
272 year (Martinez-Urtaza and Liebana, 2005; Maurer et al., 2015).

273

Conclusions

274

275 WGS technology is a valuable tool for sequencing the complete genomes of bacteria
276 and it provides insightful data into the bacterial genome. This work demonstrated that the AMR
277 genotype detected using WGS data can effectively predict the phenotypic AMR characteristics
278 with high accuracy. Furthermore, the genomic association among highly clonal *Salmonella*
279 strains could be explored using core genome data. The cgMLST scheme gave the high
280 resolution for classifying highly clonal strains of serovar Rissen. The cgMLST analysis of the
281 serovar Rissen isolates studied here provided evidence that isolates from different stages of the
282 pork production supply chain were very closely related. These findings highlight the
283 importance of stringent prevention and control measures in the pork production process to
284 reduce *Salmonella* contamination of the food chain.

285

286

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291 isolates.

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293

294

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438

439 **Table legends**

440 **Table 1:** The *Salmenella enterica* serovar Rissen isolates used in this study

441 **Table 2:** The percentage of antimicrobial resistance genotypes and phenotypes for eight

442 antibiotic groups

443 **Table 3:** Ranking of the three of the most frequent multidrug resistance (MDR) profiles,

444 plasmid replicons and *Salmonella* pathogenicity islands (SPIs) for all serovar Rissen isolates

445 from different sources.

446 **Table 4:** Sensitivity and specificity of antimicrobial resistance (AMR) genotype predictions of

447 AMR phenotypes for all 38 serovar Rissen isolates in the study

448 **Figure titles and legends:**

449 **Figure 1:** The minimum spanning tree of serovar Rissen isolated from different sources in
450 Chiang Mai and Lamphun provinces (2011 to 2014). The tree was generated by using the core
451 genome MLST scheme in BioNumerics software. The numbers on the connecting lines
452 illustrate the number of loci differing between each isolate or/ and complexes. Colors of the
453 circles indicate the different isolation sources: the isolates from fresh markets (red); the isolates
454 from pig farms (green); and the isolates from pig slaughterhouses (blue). The clusters of isolates
455 are represented by the color on the outer border of each cluster: purple cluster (major cluster),
456 follow by brown cluster, pink cluster, yellow and grey cluster.

457 **Table 1:** The *Salmenella enterica* serovar Rissen isolates used in this study

ID	Locations	Sources	Steps	Isolation date	ST	Antimicrobial Resistance Patterns
R01	Market01	Pork	-	26-Sep-14	469	AMP,C,S,SXT,TE
R02	Market02	Pork	-	25-Oct-14	469	AMP,C,S,SXT,TE
R03	Market03	Pork	-	26-Sep-14	469	AMP,C,S,SXT,TE
R04		Pork	-	06-Jul-14	469	AMP,C,S,SXT,TE
R05	Farm01	Feces (Pig 24 weeks)	-	22-Aug-11	469	AMP,C,S,SXT,TE
R06		Floor	-	05-Sep-11	469	AMP,S,SXT,TE
R07	Farm02	Boots	-	03-Jul-12	469	AMP,SXT,TE
R08		Boots	-	03-Jul-12	469	AMP,SXT,TE
R09	Farm03	Feces (Pig 12 weeks)	-	15-Jun-12	469	All susceptible
R10		Feces (Pig 18 weeks)	-	15-Jun-12	469	AMP,C,SXT
R11		Feces (Pig 18 weeks)	-	05-Jun-12	469	AMP,S,TE
R12	Farm04	Boots	-	25-May-12	469	AMP,TE
R13	Farm05	Feces (Pig 24 weeks)	-	12-Jun-12	469	AMP,S,SXT,TE
R14		Floor	-	25-Oct-11	469	All susceptible
R15	Farm06	Feces (Pig 8 weeks)	-	08-Nov-11	469	AMP,C,S,SXT
R16		Feces (Pig 12 weeks)	-	08-Nov-11	469	AMP,C,S,SXT
R17	Slaughterhouse01	Worker hands (after)	Cutting & Dressing	19-May-13	469	AMP,C,S,SXT,TE
R18		Carcass	Chilling	19-May-13	469	AMP,C,S,SXT,TE
R19		Carcass	Chilling	19-May-13	469	AMP,C,S,SXT,TE
R20		Carcass	Splitting	19-May-13	469	AMP,C,S,SXT,TE
R21		Worker hands (after)	Splitting	19-May-13	469	AMP,S,SXT,TE
R22		Knife (after)	Splitting	19-May-13	469	AMP,C,S,SXT,TE
R23		Worker hands (after)	Splitting	19-May-13	469	AMP,S,SXT,TE
R24		Floor (Before)	Lairage	19-May-13	469	AMP,S,SXT,TE
R25		Carcass	Washing	09-Jun-13	469	AMP,S,SXT,TE
R26		Carcass	Washing	09-Jun-13	469	AMP,S,SXT,TE
R27		Knife (After)	Dehairing	30-Jun-13	469	AMP,C,S,SXT,TE
R28		Floor (Before)	Lairage	30-Jun-13	469	AMP,SXT,TE
R29		Floor (After)	Lairage	30-Jun-13	469	AMP,S,SXT,TE
R30		Floor (After)	Lairage	30-Jun-13	469	AMP,C,S,SXT,TE
R31	Slaughterhouse02	Feces	Evisceration	23-Jun-13	469	TE
R32		Carcass	Splitting	23-Jun-13	469	TE
R33		Knife (After)	Dehairing	23-Jun-13	469	TE
R34		Knife (After)	Bleeding	23-Jun-13	469	TE
R35	Slaughterhouse03	Worker hands (after)	Cutting & Dressing	26-May-13	469	TE
R36		Knife (After)	Dehairing	26-May-13	469	TE
R37		Mesenteric lymph node	Evisceration	23-Jul-13	469	AMP,TE
R38		Cage	Transportation	04-Aug-13	469	AMP,S,SXT,TE

458

459 **Table 2:** The percentage of antimicrobial resistance genotypes and phenotypes for eight

460 antibiotic groups

Antimicrobial groups			Markets (%)	Farms (%)	Slaughterhouses (%)
Aminoglycoside	Genotype	*aadA1*	10.53	26.32	39.47
		aadA2	10.53	23.68	42.11
		aph3	0.00	2.63	0.00
		aph6	0.00	0.00	2.63
		strA	0.00	0.00	2.63
	Phenotype[a]	S	10.53	15.79	36.84
Beta-lactam	Genotype	bla_{TEM-1B}	10.53	26.32	47.37
	Phenotype[a]	AMP	10.53	26.32	42.11
Phenicols	Genotype	*cmlA*	0.00	15.79	15.79
		cml	10.53	7.89	23.68
		floR	2.63	0.00	2.63
	Phenotype[a]	C	10.53	10.53	18.42
Quinolone	Genotype	*qnrS1*	2.63	0.00	2.63
	Phenotype[a]	CIP	0.00	0.00	0.00
		NA	0.00	0.00	0.00
		NOR	0.00	0.00	0.00
Sulfonamide-Trimethoprim	Genotype	*sul1*	10.53	13.16	31.58
		sul2	2.63	0.00	2.63
		sul3	10.53	23.68	44.74
		dfrA12	10.53	23.68	44.74
	Phenotype[a]	SXT	10.53	21.05	39.47
Tetracycline	Genotype	tet(A)	10.53	23.68	55.26
		tet(M)	2.63	0.00	2.63
	Phenotype[a]	TE	10.53	18.42	57.89

461 [a] **Abbreviation of antimicrobial agents:** S (Streptomycin 10 µg), AMP (Ampicillin 10 µg), C

462 (Chloramphenicol 30 µg), CIP (Ciprofloxacin 5 µg), NA (Nalidixic acid 30 µg), NOR (Norfloxacin 10

463 µg), SXT (Trimethoprim-Sulfamethoxazole 1.25/23.75 µg), and TE (Tetracycline 30 µg)

464 **Table 3:** Ranking of the three of the most frequent multidrug resistance (MDR) profiles,
465 plasmid replicons and *Salmonella* pathogenicity islands (SPIs) for all *S.* Rissen isolates from
466 different sources.

Ranking	Total (n=38)	Fresh Markets (n=4)	Farms (n=12)	Slaughterhouses (n=22)
MDR pattern				
1	AMP,C,S,SXT,TE (31.58)	AMP,C,S,SXT,TE (10.53)	AMP,S,SXT,TE (5.26); AMP,C,S,SXT (5.26); AMP,SXT,TE (5.26)	AMP,C,S,SXT,TE (18.42); AMP,S,SXT,TE (18.42)
2	AMP,S,SXT,TE (23.68)	-	AMP,C,S,SXT,TE (2.63); AMP,C,SXT (2.63); AMP,S,TE (2.63); AMP,TE (2.63)	AMP,SXT,TE (2.63); AMP,TE (2.63)
3	AMP,SXT,TE (7.89)	-	-	-
Plasmid replicons				
1	IncFIB(K) (18.42)	IncFIA(HI1) (7.89)	IncFIIS (7.89)	IncFIB(K) (15.79)
2	IncFIA(HI1) (15.79)	IncFIB(K) (2.63)	IncFIA(HI) (5.26)	IncFIIS (5.26)
3	IncFIIS (13.16)	-	IncX1 (2.63)	IncFIA(HI) (2.63)
SPIs				
1	SPI-3 (100); SPI-12 (100)	SPI-3 (10.53); SPI-8 (10.53); SPI-12 (10.53)	SPI-3 (31.58); SPI-12 (31.58)	SPI-3 (57.89); SPI-12 (57.89)
2	SPI-8 (63.16)	SPI-5 (7.89)	SPI-5 (15.79)	SPI-8 (39.47)
3	SPI-5 (55.26)	SPI-1 (5.26)	SPI-8 (13.16)	SPI-2 (31.58); SPI-5 (31.58)

467

468 **Table 4:** Sensitivity and specificity of antimicrobial resistance (AMR) genotype predictions of AMR phenotypes for all 38 serovar Rissen isolates in

469 the study

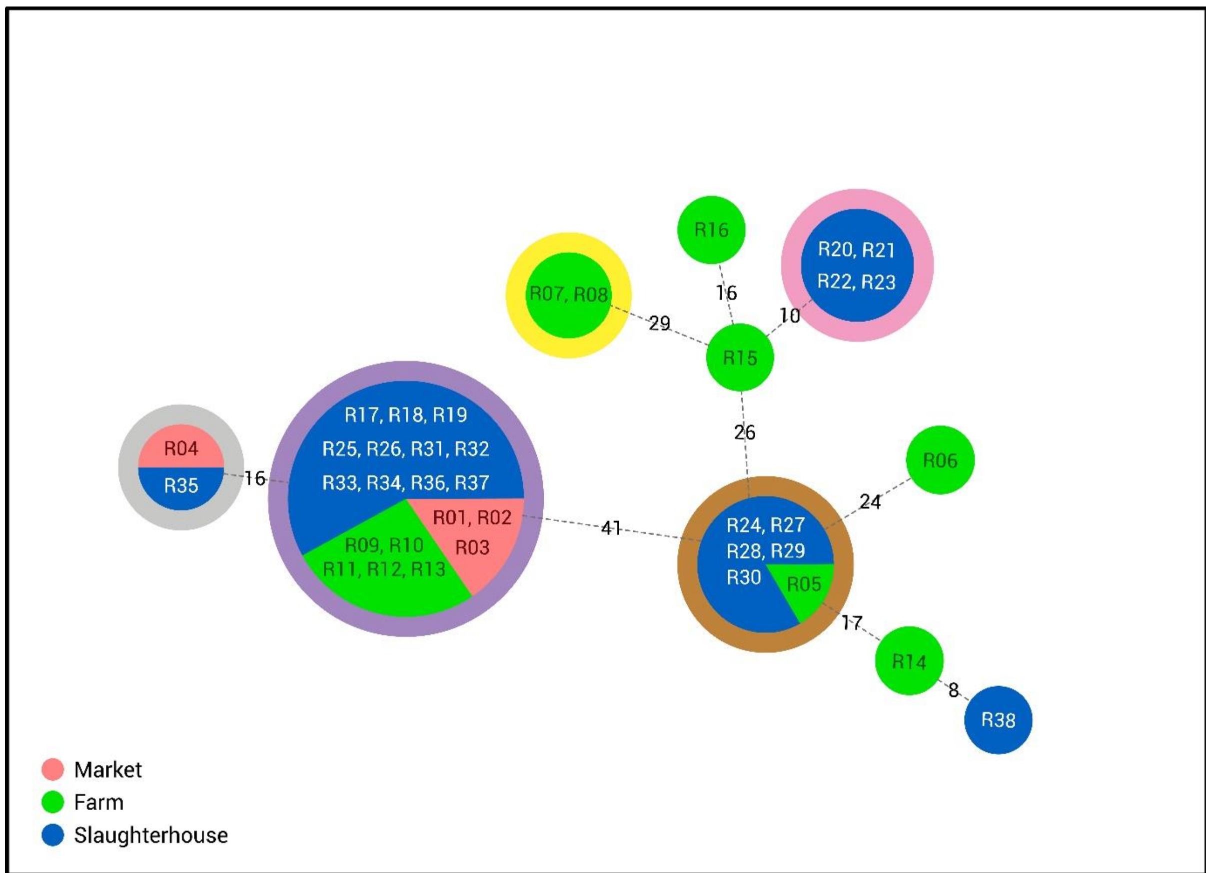
	Phenotype: Resistance		Phenotype: Susceptible		Sensitivity (%)	Specificity (%)	ROC[b] Area	Accuracy (%)
Antimicrobial[a]	WGS: AMR gene positive	WGS: AMR gene negative	WGS: AMR gene positive	WGS: AMR gene negative				
AMP	30	0	2	6	100.00	75.00	0.88	94.70
C	14	1	5	18	93.33	78.26	0.87	84.20
S	24	0	4	10	100.00	71.43	0.85	89.50
SXT	27	0	3	8	100.00	72.73	0.89	92.10
TE	31	2	1	4	93.94	80.00	0.95	92.10
Average					97.45	75.48		90.52

470 [a] AMP (Ampicillin, 10 µg), C (Chloramphenicol, 30 µg), S (Streptomycin, 10 µg), TE (Tetracycline, 30 µg) and SXT (Trimethoprim-

471 Sulfamethoxazole, 1.25/23.75 µg)

472 [b] ROC = Receiver operating characteristic

473



474 **Figure 1:** The minimum spanning tree of serovar Rissen isolated from different sources in
475 Chiang Mai and Lamphun provinces (2011 to 2014). The tree was generated by using the core
476 genome MLST scheme in BioNumerics software. The numbers on the connecting lines
477 illustrate the number of loci differing between each isolate or/ and complexes. Colors of the
478 circles indicate the different isolation sources: the isolates from fresh markets (red); the isolates
479 from pig farms (green); and the isolates from pig slaughterhouses (blue). The clusters of isolates
480 are represented by the color on the outer border of each cluster: purple cluster (major cluster),
481 follow by brown cluster, pink cluster, yellow and grey cluster.

482