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

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Highlights

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

Abstract

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

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

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).

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

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

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

Materials and Methods

Bacterial strains and molecular typing

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

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

Whole Genome Sequencing

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

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

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

(*cml*A1, *cml*, and *flo*R), sulfonamide (*sul*1, *sul*2, and *sul*3), tetracycline (*tet*(A) and *tet*(M)), and trimethoprim (*dfr*A12) resistance genes. The *Salmonella* pathogenicity islands (SPI), MLST sequence type, and plasmid were examined by SPIFinder 1.0 (https://cge.cbs.dtu.dk/services/SPIFinder/) (Kozyreva et al., 2016), MLST 2.0 (https://cge.cbs.dtu.dk/services/MLST/), and PlasmidFinder 2.0 (https://cge.cbs.dtu.dk/services/PlasmidFinder/) (Carattoli et al., 2014; Larsen et al., 2012).

The correlation between AMR genotype and phenotype

The sensitivity of AMR genotype prediction was calculated by the number of resistance phenotypes divided by the total number of isolates exhibiting AMR phenotypes. Specificity was also calculated by dividing the number of the susceptible genotypes by the total number of isolates with susceptible phenotypes. The receiver operating characteristic (ROC) curves were analysed to determine antimicrobial resistant phenotype of corresponding genes. The area under the ROC curve (AUC) was calculated to evaluate the accuracy of the prediction.

Analysis by core genome multilocus sequence typing (cgMLST)

The cgMLST analysis was conducted using BioNumerics software version 7.6.3 (Applied Maths, Sint-Martens-Latem, Belgium). The wgMLST schema in the software consists of a total of 15,874 loci from 199 of publicy available *Salmonella enterica* reference genomes. The cgMLST analysis was restricted to loci with ≥ 80% homology in ≥ 95% of the isolates (2,516 loci). The minimum spanning tree (MST) was generated using the algorithm for clustering categorical data.

Results

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

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

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

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

Genotype predictions of the AMR phenotype

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

Core genome and whole genome MLST analysis

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

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

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

Discussion

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

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

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

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

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

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

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

Conclusions

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

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438

Table legends

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

Table 2: The percentage of antimicrobial resistance genotypes and phenotypes for eight antibiotic groups

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

Table 4: Sensitivity and specificity of antimicrobial resistance (AMR) genotype predictions of 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
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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	$bla_{TEM\text{-}1B}$	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

[a] **Abbreviation of antimicrobial agents:** S (Streptomycin 10 µg), AMP (Ampicillin 10 µg), C (Chloramphenicol 30 µg), CIP (Ciprofloxacin 5 µg), NA (Nalidixic acid 30 µg), NOR (Norfloxacin 10 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)

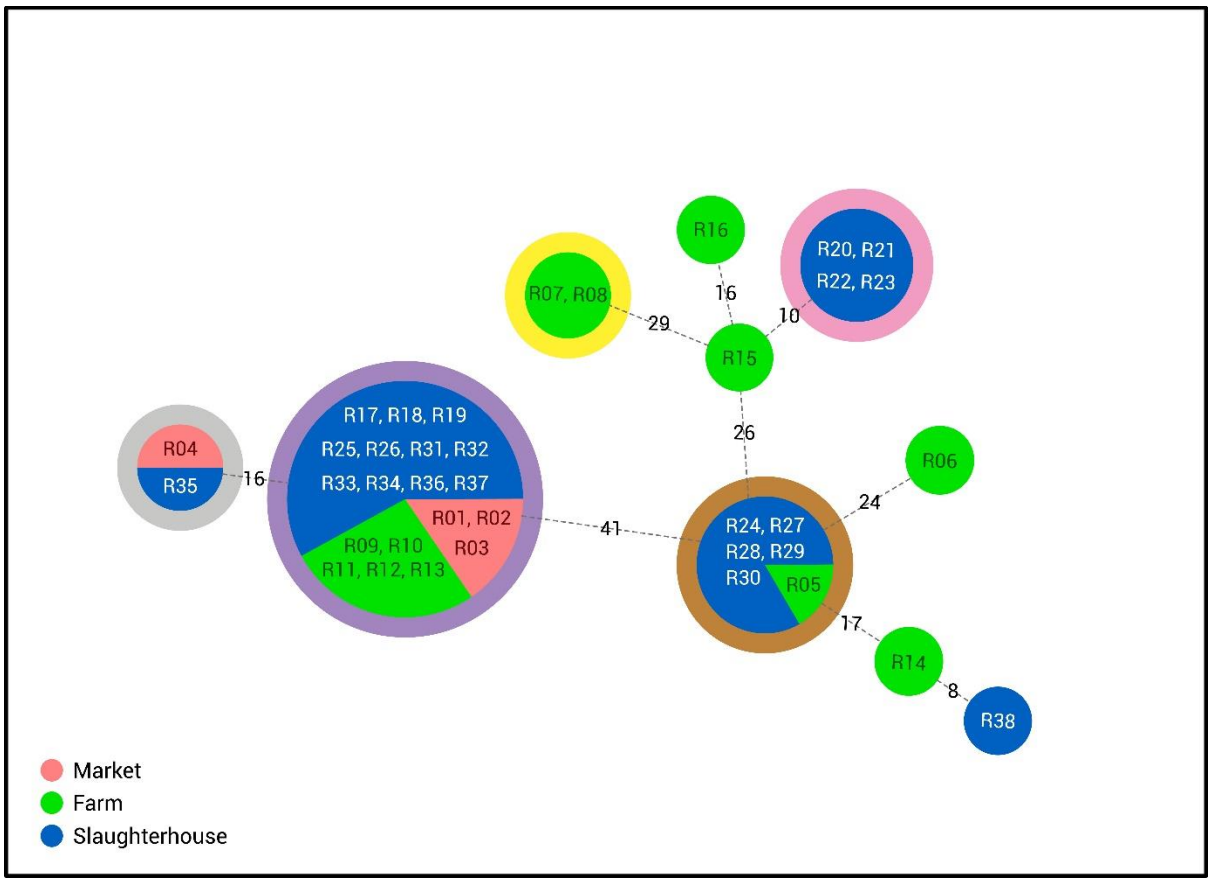
467

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

[a] AMP (Ampicillin, 10 µg), C (Chloramphenicol, 30 µg), S (Streptomycin, 10 µg), TE (Tetracycline, 30 µg) and SXT (Trimethoprim-Sulfamethoxazole, 1.25/23.75 µg)

[b] ROC = Receiver operating characteristic



473

474 **Figure 1:** The minimum spanning tree of serovar Rissen isolated from different sources in
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