1 **Non-serotype 2 isolates from healthy pigs are a potential zoonotic reservoir of *Streptococcus suis***
2 **genetic diversity and antimicrobial resistance**

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25 **Running title:** MDR *S. suis* from healthy pigs

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28 **Significance statement**

29 The zoonotic pathogen *Streptococcus suis* causes respiratory disease in pigs and is among the most
30 common causative agents of human clinical bacterial meningitis in SE Asia. We collected isolates
31 from farmed healthy pigs in Northern Thailand, representing a source population from which invasive
32 isolates have recently emerged – linked to the pork production industry. Pangenome characterisation
33 of the isolates revealed a reservoir of genetic diversity and antimicrobial resistance suggesting that
34 One Health approaches may be beneficial in tackling the increase in antimicrobial resistance.

36 **Summary**

37 *Streptococcus suis* is a leading cause of bacterial meningitis in SE Asia, with frequent zoonotic
38 transfer to humans associated with close contact with pigs. A small number of invasive lineages are
39 responsible for endemic infection in the swine industry causing considerable global economic losses.
40 A lack of surveillance and a rising trend in clinical treatment failure has raised concerns of growing
41 antimicrobial resistance (AMR) among invasive *S. suis*. The source-sink dynamics between healthy
42 and disease isolates is poorly understood and, in this study, we sample and sequence a collection of
43 isolates predominantly from healthy pigs in Chiang Mai province, Northern Thailand. Pangenome
44 comparisons with a selection of invasive serotype 2 isolates identified increased genetic diversity and
45 more frequent AMR carriage in isolates from healthy pigs. Multiple antimicrobial resistance genes
46 were identified conferring resistance to aminoglycosides, lincosamides, tetracycline and macrolides.
47 All isolates were non-susceptible to three or more different antimicrobial classes, and 75% of non-
48 serotype 2 isolates were non-susceptible to 6 or more classes (compared to 37.5% of serotype 2
49 isolates). Antimicrobial resistance genes were found on integrative and conjugative elements (ICE)
50 previously observed in other species, suggesting mobile gene pool which can be accessed by invasive
51 disease isolates.

53 **Keywords:** *Streptococcus suis* / antimicrobial resistance / zoonosis / horizontal gene transfer / mobile
54 elements / one health / gene pool transmission / meningitis

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56 **Background**

57 More than half the world's pork meat is produced in SE Asia, and China alone is home to nearly half
 58 the world's live pigs. The United Nations' Food and Agricultural Organization estimated that China
 59 produces around half of the billion pigs reared worldwide (Gilbert et al., 2018). This massive increase
 60 in agricultural intensification has brought significant challenges in animal welfare, including infection
 61 control. Among the most common infections to Asian herds is a respiratory disease caused by
 62 *Streptococcus suis* (VanderWaal and Deen, 2018). Infection with *S. suis* occurs mainly in piglets and
 63 growing pigs and can lead to septicemia with sudden death, arthritis, endocarditis, meningitis
 64 (Dutkiewicz et al., 2017; Gottschalk and Segura, 2019; Segura, 2020). *S. suis* infections accounted for
 65 a loss of over US\$11 million to the pork industry in Thailand alone in 2019 (Rayanakorn et al., 2020).
 66 This expanded niche for *S. suis* has provided opportunities for zoonotic infection, which are
 67 frequently reported worldwide following increased exposure to pigs, often in farm workers,
 68 slaughterhouse workers, and butchers (Goyette-Desjardins et al., 2014; van Samkar et al., 2015;
 69 Dutkiewicz et al., 2017). However in SE Asia, particularly in Northern Thailand, where there is a
 70 tradition of consuming raw pork dishes, *S. suis* infection is one of the most common causative agents
 71 of clinical bacterial meningitis (Takeuchi et al., 2017; Rayanakorn et al., 2019).

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73 Human zoonotic *S. suis* infections predominantly arise from a single virulent lineage, thought to have
 74 emerged in the 1920s alongside the intensification of the pork production industry. However, no
 75 consistent genomic differences between pig and human disease isolates have been observed (Weinert
 76 et al., 2015). This may be related to the fact that isolates from healthy (asymptomatic) pigs have not
 77 been well studied but it is known that disease-associated isolates have fewer genes overall but more
 78 that encode putative virulence factor (Weinert et al., 2019; Murray et al., 2021). Serotyping of the *S.*
 79 *suis* capsular polysaccharides is often used in epidemiological studies, with 29 *S. suis sensu stricto*
 80 serotypes described to date (Athey et al., 2016a; Segura et al., 2016). *S. suis* serotype 2 is the most
 81 virulent and is frequently isolated from diseased pigs and human clinical cases (Hughes et al., 2009;
 82 Okura et al., 2016); however, non-serotype 2 isolates (often isolated from healthy pigs) represent an

83 extensive reservoir of genetic diversity (Zhang et al., 2011; Baig et al., 2015; Okura et al., 2019;
84 Stevens et al., 2019).

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86 Widespread use of antimicrobial drugs in the pig production industry has driven an increase in
87 antimicrobial resistance (AMR) (Van Boeckel et al., 2015; WHO, 2017). Imprudent use of colistin in
88 pork production as a growth enhancer (since the 1970s) encouraged the development of resistance in
89 *E. coli* (and other gram-negative bacteria), which has diminished the effectiveness of antibiotics used
90 in human medicine (Liu et al., 2016; Delannoy et al., 2017; Patchanee et al., 2020a). Furthermore,
91 there is a rising trend in multi-drug resistant (MDR) zoonotic pathogens, such as *Salmonella*, that pose
92 a significant public health threat (Prasertsee et al., 2019; Patchanee et al., 2020b; Tadee et al., 2021).
93 Regulation of veterinary use of antibiotics is difficult in low- and middle-income countries, which
94 consequently have some of the highest AMR levels (Nguyen et al., 2016). For example, in Thailand
95 alone, infections with antimicrobial-resistant bacteria are estimated to cause up to 38,000 human
96 deaths each year (Pumart P, 2012).

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98 A lack of surveillance and rise in clinical treatment failure has raised concerns of growing
99 antimicrobial resistance among invasive *S. suis* (Pathanasophon et al., 2013). Furthermore, the source-
100 sink dynamics among commensal and disease-causing isolates are poorly understood. In this study,
101 we sample and sequence a collection of isolates predominantly from healthy pigs in Chiang Mai
102 province, Northern Thailand. Pangenome comparisons with a selection of invasive serotype 2 isolates
103 identified increased genetic diversity and more frequent AMR carriage in isolates from healthy pigs.
104 Antimicrobial resistance genes were found on integrative and conjugative elements (ICE) previously
105 observed in other species, suggesting mobile gene pool which can be accessed by invasive disease-
106 causing isolates.

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Results

All *S. suis* samples collected from healthy pigs in Chiang Mai province, Thailand were identified by PCR (**Table S1**) as non-serotype 2 isolates. From the 138 isolates we collected, 25 were randomly selected for whole genome sequencing. An additional 11 isolates from lab archives, previously collected from Chiang Mai were added to the dataset to include representative isolates from pig disease and invasive human infection. In total, the dataset used consisted of 36 isolates, of which 8 isolates (22.2%) were serotype 2, including isolates from human clinical cases (n=2), diseased pigs (n=2), and healthy pigs (n=4) and 28 isolates (77.8%) of non-serotype 2 *S. suis* from healthy pigs (**Figure 1A; Table S2**).

Non-serotype 2 isolates are a reservoir of antimicrobial resistance

No non-serotype 2 isolates were responsible for disease in either pigs or humans. A maximum-likelihood phylogeny constructed from a concatenated gene-by-gene core genome alignment (1,348 genes) revealed a highly structured population (**Figure 1B; Supplementary file S1**). Serotype 2 isolates clustered together, including the previously described sequence types ST-1, ST-28, ST-104 and ST-105. Pairwise average nucleotide identity (ANI) comparisons suggested that non-serotype 2 isolates (75.1% identical) were more diverse than serotype 2 isolates (98.1% identical) in the core genome (**Figure 2AB**). This was supported by (pairwise) clustering of the core and accessory genome using PopPUNK (Lees et al., 2019), which identified divergence in the accessory genomes of the serotype 2 isolates (**Figure 2C**). Together the pangenome of all 36 isolates comprised 5,004 gene clusters, with 1,348 core genes present in at least 95% of isolates representing ~27% of the pangenome; or ~68% of the average *S. suis* genome (1,993 ORFs in BM407; **Figure 2D; Table S3**). Typically, invasive serotype 2 isolates have smaller genomes but contain more virulence-related genes (Weinert et al., 2015). In our dataset, this was also true with serotype 2 isolates having smaller genomes on average (**Table S2**), and the virulence associated *pilB* gene was found in 75% (n=3 of 4) of invasive isolates, but only 7% of isolates from healthy pigs (n=2 of 28) (**Figure 1B; Table S4**).

Widespread AMR determinants in S. suis isolates from healthy pigs

We scanned all 36 genomes for known determinants of antimicrobial resistance (AMR) through nucleotide comparisons ($\geq 70\%$ sequence identity) with the NCBI database (Coordinators, 2018; Bortolaia et al., 2020) and identified 18 resistance genes from 7 different antimicrobial classes (**Figure 1B; Table S5**). Loci conferring putative resistance to aminoglycosides (*aadE*, *ant(6)-Ia*, *aph(3')-III* and *spw*); macrolides (*erm(A)*, *erm(B)*, *erm(T)*, *mef(A)* and *msr(D)*); lincosamides (*lsa(E)*, *lnu(B)* and *lnu(C)*); tetracycline (*tet(w)*, *tet(L)* and *tet(O)*); oxazolidinone (*optrA*); nucleoside (*sat4*) and chloramphenicol (*catA8*) were found in 32 isolates (89%). On average, fewer antibiotic resistance genes were identified in the serotype 2 isolates (5 genes) compared to non-serotype 2 isolates (18 genes; **Table S5**). All 18 of the resistance genes were detected in the non-serotype 2 isolates from healthy pigs, but only five of the potential AMR genes *spw*, *lsa(E)*, *erm(B)*, *lnu(B)*, and *tet(O)* were found in the eight serotype 2 isolates, including *tet(O)* which was present in all serotype 2 isolates. At least one antimicrobial resistance gene from three or more antimicrobial classes was found in 21 out of 36 isolates (58%), and only one out of these was a *S. suis* serotype 2 isolate.

Evidence of mobility of AMR genes among S. suis from healthy pigs

Comparison of nucleotide sequences from all the genomes with the PlasmidFinder database (Carattoli et al., 2014) identified loci identified on six putative integrative and conjugative elements (ICE), including pLFE1, pBM407, pAMbeta, Col440II, pLW043, and pCW7 (**Figure 1B; Table S6**). All putative ICE elements were identified in non-serotype 2 isolates (39%; 11 of 28). Two of these ICE elements have previously been characterized in invasive *S. suis*, pBM407 (accession: FM252033) and pAMbeta (accession: AE002565.1). The pBM407 plasmid described in *S. suis* contained AMR genes conferring resistance to tetracycline (*tetO*, *tetL*), chloramphenicol (acetyltransferase), erythromycin (*ermB*) and a dihydrofolate reductase (Holden et al., 2009). However, plasmids from two different isolates with variation in gene content hint at an underlying diversity - and this potential composite architecture was evidenced by differences in the AMR gene complement (Holden et al., 2009). All serotype 2 isolates contain the *tetO* locus, and 75% (6 of 8) contain the *ermB* locus which are described as members of the pBM407 ICE element, but no other pBM407 genes are identified by this method (**Table S6**). Additional plasmids not previously described in *S. suis* were also identified using

MOB-suite, which compares genome sequences with all described plasmids in the NCBI database (Table S7; (Robertson and Nash, 2018; Robertson et al., 2020).

Widespread antimicrobial resistance in non-serotype 2 isolates

Disk diffusion assays were used to determine antimicrobial susceptibility of the isolates to 18 antimicrobial agents, from 9 antimicrobial categories. Most isolates were highly susceptible to linezolid (100%; n=36), amoxicillin-clavulanic acid (97%; n=35), ceftiofur (94%; n=34), amoxicillin (83%; n=30) and ampicillin (81%; n=29). High levels of resistance were observed against lincomycin (100%; n=36), clindamycin (97%; n=35), tetracycline (92%; n=33), doxycycline (92%; n=33), kanamycin (89%; n=32), oxytetracycline (83%; n=30), erythromycin (69%; n=25) and gentamycin (31%; n=11) (Table 1). There was a statistically significant difference in antimicrobial susceptibility between *S. suis* serotype 2 and non-serotype 2 isolates for gentamycin (*p*-value: 0.037), chloramphenicol (*p*-value: 0.010), and penicillin G (*p*-value: 0.001; Pearson's Chi-square test and Fisher's exact test) (Table 1).

Multi-drug resistance (MDR) is defined as an isolate that is non-susceptible to at least one antimicrobial agent from three different antimicrobial categories (Sweeney et al., 2018). In this study, we will consider all non-susceptible isolates as resistant. All 36 *S. suis* isolates were resistant to three or more antibiotic classes (Figure 3A; Table 1). Most (87.5%; 7 of 8) serotype 2 isolates were resistant to four and five antimicrobial categories; while three quarters (21 of 28) of non-serotype 2 isolates were resistant to 6, 7 and 8 antimicrobial categories (46.4, 25 and 3.6%, respectively; Figure 3B).

Diverse AMR profiles in *S. suis* isolates from healthy pigs

Increased diversity in the core, accessory, and plasmid content of non-serotype 2 isolates was associated with increased AMR conferred by 21 different antimicrobial resistance gene (ARG) profiles (A–U; Figure 3A; Table 2). The most common ARG pattern included the *erm(B)* and *tet(O)* genes (13.9%), followed by *aadE/erm(B)/tet(O)* and *ant(6)-Ia /erm(B)/mef(A)/msr(D)/tet(W)* gene

patterns (8.3%). All putative resistance genes were absent in 4 non-serotype 2 strains (11.1%). Non-serotype 2 isolates demonstrated greater variation in ARG content, with only three resistance gene patterns (B, C and P) found exclusively in serotype 2 isolates.

Phenotypic and genotypic concordance in antimicrobial resistance

When we compared phenotypic non susceptibility (zones of inhibition) with the presence of specific ARGs, often there was no clear correlation (**Table 3; Figure S1**). There was little correlation between gentamycin, kanamycin, lincosamide, clindamycin resistance and the presence of any specific ARG. Widespread resistance to tetracycline (97.2% non susceptible; **Table 1**), doxycycline and oxytetracycline correlated with the presence of ARGs, including *tet(O)*, *tet(W)* and *tet(L)* (OR > 1; **Table 3**). The strongest link between phenotype and ARG was observed for erythromycin resistance and the presence of *erm(B)* (OR= 32.5, $p = 0.002$; **Table 3**). Phenotypic resistance to erythromycin correlated well with the ARGs *erm(A)*, *erm(T)*, *mef(A)* and *msr(D)*; and 72.2% of erythromycin resistant isolates contained the *erm(B)* gene (**Table 3; Figure S1**). There were not enough resistant isolates to properly assess the correlation between the presence of genes linked to resistance to chloramphenicol (n=1) and florfenicol (n=1) and none of the isolates were resistant to the first generation oxazolidinones, linezolid, despite identification of the corresponding *optrA* resistance gene (**Table 3; Figure S1**).

Discussion

S. suis were cultured and identified from 18.6% of pigs swabbed in this study (138 of 760 samples), which is within the range previously reported for the prevalence in farmed pigs and slaughterhouses in the same area of Thailand (Padungtod et al., 2010; Kongkaew et al., 2012). This level of prevalence was significantly lower than the *S. suis* prevalence previously reported in pigs from other provinces in Northern Thailand, such as Lampang (64.8%) and Phayao (61.4%) (Pathanasophon et al., 2013). These and other studies in Northern Thailand reported high prevalence of serotype 2 (5.6- 43%) and serotype 7 (8.2-14.3%) isolates (Padungtod et al., 2010; Thongkamkoon et al., 2017). However, we mainly identified non-serotype 2 isolates, with only a single isolate typed as serotype 9 and no serotype 2 isolates identified during this survey. This variation is likely due to differences in sampling, as we prioritised collection from healthy pigs. Invasive disease isolates have shown biogeographical variation, and with competition and serotype replacement noted among virulent *S. suis* serotypes (Flores et al., 1993; Hughes et al., 2009; Hadjirin et al., 2020). Serotype 9 is most common in diseased pigs from Europe and has a low pathogenic potential in humans. However, a rare case of serotype 9 infection in humans has recently been reported in Thailand (Goyette-Desjardins et al., 2014; Kerdsin et al., 2017).

Difficulties in (capsule) serotyping *S. suis* (*sensu lato*) isolates, where previously typed *S. suis* isolates are now designated as other *Streptococcus* species hint at an ambiguous species designation and variation in non-serotype 2 isolates (Prüfer et al., 2019; Hatrongjit et al., 2020). This is further supported by characterisation of divergent *S. suis* isolates by whole genome sequencing (Baig et al., 2015). The extent to which these represent stable lineages is unclear, with the rate of lineage turnover in *S. suis* seldom investigated (Calland et al., 2020; Hadjirin et al., 2020). We identified increased variation in the core and accessory genomes of non-serotype 2 isolates (**Figures 1 and 2**). Serotype 2 isolates are typically found to have smaller genomes than non-invasive isolates (Baig et al., 2015; Weinert et al., 2015) and our collection of mostly non-invasive non-serotype 2 isolates were consistently larger (with more genes; **Table S2**) than the *S. suis* reference genome (BM407: 2,170,810 bp) and selected invasive isolates (**Table S2**). Despite smaller genomes, these invasive isolates also

tend to carry more virulence-related genes and all serotype 2 isolates in our collection carried the *pilB* gene, which is associated with the brain cell invasion required to cause meningitis in humans and pigs (Maisey et al., 2007). It has been suggested that this reduction in genome size may be due to gene loss, including core metabolism genes for nutrients that can be scavenged from the host; and a streamlining of functional/redundant elements (Weinert and Welch, 2017; Murray et al., 2021).

A plug-and-play theory of bacterial accessory genomes (Young, 2016; McInerney et al., 2017; Sheppard et al., 2018), where diversity in bacterial phenotypes can be conferred by a mobile pool of genes that are readily gained and lost, enables the acquisition of rapid adaptive genomic changes that can be spread through the population via recombination (Redondo-Salvo et al., 2020). Host switching and zoonotic infection complicate analyses of source and sink dynamics and attribution of AMR elements (Dearlove et al., 2016; Mourkas et al., 2019). Here, we focus primarily on the potential reservoir of infection and characterize variation in the gene pool from which invasive disease isolates have arisen. Where resistance is conferred by a single (or few) nucleotide substitution(s), it is impossible to tell from sequence data if HGT or point mutation were responsible (Zhao et al., 2016; Bortolaia et al., 2020). For other classes of antibiotics, the literature provides clear evidence for HGT of genes (Florez-Cuadrado et al., 2017; Wang et al., 2018; Patchanee et al., 2020a; Redondo-Salvo et al., 2020). For example, the pBM407 plasmid characterised in the pBM407 *S. suis* reference genome mobilizes *tetO*, *tetL*, *emrB*, *cat* and *dfp* genes between isolates (Holden et al., 2009; Hoa et al., 2011). Including these putative tetracycline resistance genes, our analyses identified 18 accessory genes associated with resistance to 7 antimicrobial classes.

We identified genes with described roles in resistance to aminoglycosides, macrolides, lincosamides, tetracycline, nucleoside, oxazolidinones, and phenicols. Most isolates were predicted to be MDR (80.6%). The most common antimicrobial resistant genes identified were associated with resistance to macrolides and tetracycline. More than 80% of isolates contained at least one gene predicted to confer macrolide resistance (Palmieri et al., 2011). The presence of *erm(B)* and *mef(A)* genes are consistent with previous studies, where *erm(B)* is strongly linked with macrolide-lincosamide-streptogramin B

(MLS_B) resistance and presented in 59-90% of macrolide-resistant *S. suis* isolates from pigs (Martel et al., 2001; Zhang et al., 2015; Tan et al., 2020). The resistant gene, *erm(T)* has been detected in *S. agalactiae*, *S. pyogenes*, and other erythromycin-resistant isolates of group D *Streptococci* (Chen et al., 2012; Zhang et al., 2015; Yongkiettrakul et al., 2019), our identification of *erm(T)* in this study suggests potential within-genus HGT.

The most common tetracycline resistance gene detected was *tet(O)* in over half of the isolates (63.9%) (**Table 3**). An alternative ribosomal protein, *tet(M)* is also often associated with tetracycline resistance in *S. suis* (Palmieri et al., 2011; Bojarska et al., 2016) but was not observed among our isolates. In addition, we detected *tet(L)* and *tet(W)* genes, which have not often been reported in *S. suis*, among non-serotype 2 isolates from healthy pigs. Corresponding phenotypic resistance to tetracycline was reported in over 90% of isolates, which is consistent with global data reporting widespread resistance to tetracycline and macrolides, likely related to the prophylactic use in agriculture (Soares et al., 2014; Yongkiettrakul et al., 2019; Mourkas et al., 2020; Tan et al., 2020). AMR may play a role in increasing numbers of treatment failures (Hughes et al., 2009; Gurung et al., 2015; Yongkiettrakul et al., 2019), and in our study, despite widespread MDR, we observed phenotypic susceptibility to all three of the recommended antimicrobials used to treat clinical *S. suis* meningitis (penicillin, ceftiofur, and ceftriaxone) (van Samkar et al., 2015; Seitz et al., 2016). However, some β -lactam resistant strains (18-27%) were found in the non-clinical strain of *S. suis* (Soares et al., 2014; Yongkiettrakul et al., 2019; Segura et al., 2020). Despite this, β -lactam usage in pig production should be closely monitored, especially where there is prophylactic use in healthy pigs.

Concordance between the presence of predicted ARGs and phenotypic resistance was poor for most antimicrobial, and we report widespread phenotypic resistance, even in the absence of a predictive resistance element (**Figure 3**). Given the enhanced genetic diversity and lack of clear characterization of this disease reservoir, it is possible that additional resistance elements have yet to be fully described. A recent study by Hadjirin et al. identified more than 20 novel *S. suis* AMR determinants (Hadjirin et al., 2021). Even in the absence of direct antimicrobial selective pressure, broad spectrum

use of antibiotics act on all bacterial species in the microbiome; and this bystander effect can confer resistance on bacterial species that are not the target of the antimicrobial treatment (Tedijanto et al., 2018; Morley et al., 2019). Enrofloxacin is widely used to treat other types of bacterial infection in the respiratory and digestive systems of livestock animals, and in our collection more than 40% of isolates were resistant to this antibiotic (Lakkitjaroen et al., 2011; Yongkiettrakul et al., 2019). Spectinomycin is often used in pig production and other livestock animals combined with lincomycin (Bosman et al., 2019; Wang et al., 2020). Clusters of AMR genes (*aadE-spw-lsa(E)-lnu(B)*) have been identified in *Staphylococci* and *Enterococci* associated with lincosamides resistance (Li et al., 2014; Huang et al., 2016). We identified the combination of spectinomycin and lincosamide resistance in one serotype 2 isolate and 2 non-serotype 2 isolates from healthy pig. Individually, we identified spectinomycin and lincosamides resistance genes in a small number of isolates, as has previously been reported in invasive *S. suis* isolates (Athey et al., 2016b; Bojarska et al., 2016).

The plasmid-borne chloramphenicol resistance gene, *catA8* (McHugh et al., 2020; Yan et al., 2020), and the *optrA* gene that confers transferable combined resistance to oxazolidinones (linezolid) and phenicols (chloramphenicol and florfenicol) (Brenciani et al., 2016; Bender et al., 2018; Zhou et al., 2019) are reported here for the first time in *S. suis*. Although phenotypic susceptibility was recorded to linezolid, the isolates were resistant to chloramphenicol and florfenicol. Recently, this gene has been found in oxazolidinone-resistant *S. suis* isolates in China (Huang et al., 2017; Du et al., 2019; Huang et al., 2019). This is further evidence of the unintended effect of broad-spectrum antimicrobials, such as the oxazolidinones linezolid and tedizolid, which are highly effective against Gram-positive bacteria (Sztanke et al., 2004) but rarely used in the pig production industry. However, florfenicol has been used in livestock animals for therapeutic purposes and there is documented transfer of plasmids carrying *optrA* between different Gram-positive bacteria (Wang et al., 2015). Twenty-one different resistance gene patterns were observed, with *erm(B)* and *tet(O)* found together in 62.5% (5 of 8) of serotype 2 isolates, as previously observed (Athey et al., 2016b). Most non-serotype 2 isolates possessed ARGs to at least three antimicrobial classes (up to seven; 22/28, 78.6%). Several genetic elements, including ICE carrying antimicrobial resistance genes such as *optrA*, *ermB*,

tetM, *tetO*, and *tetW* have been reported in *S. suis* (Holden et al., 2009; Athey et al., 2016b), however plasmid elements were found only in non-serotype 2 isolates in this study.

Conclusion

We collected isolates from 760 healthy pigs reared in the pork industry in Northern Thailand. Through comparison of 36 whole genome sequences, we identified increased genetic diversity in these non-serotype 2 carriage isolates, from which the more invasive and pathogenic serotype 2 isolates emerge. Corresponding diversity was also seen in the breadth and diversity of AMR determinants which conferred increased phenotypic non-susceptibility. This genetically diverse reservoir of *S. suis* pose a public health risk with the potential for transmission to more invasive isolates, broadening their spectrum of antimicrobial resistance. Extensive phenotypic resistance is observed to antimicrobials that are not typically used to treat this infection. This can be partly explained by the co-occurrence of resistance genes on ICEs. However, little phenotypic resistance was observed to β -lactams, which remain the prescribed antimicrobial for *S. suis* infection in Thailand. Continued surveillance and more stringent control of antimicrobial usage within the pork industry will be necessary to monitor a growing AMR threat in *S. Suis*.

Methods

Ethical approval

This study was carried out according to the guidelines for the care and use of laboratory animals (National Research Council, 2010). The study protocol was approved by the Faculty of Veterinary Medicine's Animal Care and Use Committee (Protocol number S24/2559).

Sample collection

Samples were collected between March and November 2015, with a total of 760 tonsil swab samples collected from 111 pig farms in 25 districts of Chiang Mai province, Thailand. All swab samples were kept in Stuart transport medium (Oxoid, UK) and transported to the laboratory at 4 °C within 24 hrs of collection.

Live fattening pigs were swabbed, and *S. suis* identified in 138 samples (18.2%). Of 138 *S. suis* isolates obtained from healthy pigs, only one isolate (0.7%) was confirmed as *S. suis* serotype 9. Meanwhile, all the remaining 137 isolates (99.3%) were negative to serotypes serotype 1/2/7/9/14 by PCR identification and classified as non-serotype 2 strains. Among 138 strains, 25 strains were randomly selected for WGS. In addition, 11 isolates were selected from laboratory archives (collected as part of another study of farmed pigs in Chiang Mai province during 2015) and sequenced for comparison between serotype 2 and non-serotype 2 isolates.

Bacterial identification and growth

Tonsil swab samples were inoculated onto 5% sheep blood agar plates (Oxoid, UK) and incubated at 37°C for 24 hours. *S. suis* isolates were identified by biochemical characterization (Quinn et al., 1994), and small (approximately 1 mm in diameter) transparent alpha- hemolysis and non-hemolysis colonies of Gram-positive cocci with negative catalase test were selected for further screening. Criteria for presumptive identification of *S. suis* included no growth on 6.5% NaCl agar, a negative Voges-Proskauer (VP) test, and production of acid in trehalose, lactose, sucrose, salicin and inulin broths, but no acid production in glycerol, sorbitol, and mannitol. A multiplex polymerase chain

reaction (PCR) using primers specific to the 16S rRNA gene was used to confirm the identification of *S. suis* and capsular gene types 1 or 14, 2 or 1/2, 7, and 9, which are the most prevalent serotypes recovered from diseased pigs, as described in **Table S1** (Wisselink et al., 2000; Wisselink et al., 2002; Marois et al., 2004).

Antimicrobial susceptibility testing

Antimicrobial susceptibility tests were performed using the disk diffusion method in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2012). Eighteen antibiotic drugs from 9 antibiotic groups were used in the test, including aminoglycoside (gentamycin, 10 µg; and kanamycin, 30 µg), lincosamides (lincomycin, 10 µg; and clindamycin, 2 µg), macrolides (erythromycin, 15 µg), tetracyclines (tetracycline, 30 µg; doxycycline, 30 µg; and oxytetracycline, 30 µg), oxazolidinone (linezolid, 30 µg), phenicols (chloramphenicol, 30 µg; and florfenicol 30 µg), beta-lactams (ampicillin, 10 µg; penicillin G, 10 units; amoxycillin, 10 µg; amoxycillin-clavulanic acid, 30 µg; and ceftiofur, 30 µg), fluoroquinolones (enrofloxacin, 5 µg) and folate inhibitors (sulfamethoxazole/trimethoprim, 1.25/23.75 µg) (Oxoid. Hamshire, UK). Diameter breakpoints were assessed according to the guidelines described in Table 1 (CLSI, 2002, 2008, 2012; Howe and Andrews, 2012; NEO-SENSITABS™, 2013; CLSI, 2017, 2018, 2020). Pearson's Chi-square test and Fisher's exact test were performed using IBM SPSS Statistics version 22 (IBM, New York, USA) to determine the difference of antimicrobial susceptibility between *S. suis* serotype 2 and non-serotype 2. The association between antimicrobial-resistant phenotype and genotype was tested by Pearson's chi-square test, and Yate's correction for continuity was applied where required. Statistically significant associations were shown as odds ratios (ORs) with 95% confidence intervals (CI). Results were considered statistically significant when a two-tailed *p*-value ≤ 0.05.

Genome sequencing and assembly

Twenty-five *S. suis* isolates from pigs with no clinical signs of *S. suis* infection (healthy pigs) were randomly selected for sequencing from the 138 recovered samples. Our collection was augmented with two archived isolates derived from tissue samples of pigs with clinical signs of *S. suis* infection

(diseased pigs) that were submitted to the Veterinary Medicine Research and Development Center (Upper Northern region) of the National Institute of Animal Health (Thailand), and a further nine isolates from the Faculty of Medicine at Chiang Mai University. In total, our collection included 32 healthy pigs, two diseased pigs, and two human clinical samples cultured from the blood of meningitis patients. All strains were cultured in Todd-Hewitt-broth at 37 °C for 18-24 hrs, and genomic DNA was extracted using the QIAamp DNA Minikit (QIAGEN®). Whole-genome sequencing (WGS) using a multiplex sequencing approach was performed on an Illumina Miseq genome sequencer (Illumina, Cambridge UK) using Nextera XT libraries and third generation MiSeq reagent kits. Paired-end short reads of 300 bp were filtered, trimmed, and assembled *de novo* with SPAdes version 3.7 (Bankevich et al., 2012). The average number of contiguous sequences (contigs) in 36 *S. suis* genomes was 160 for an average total assembled sequence size of 2.22 Mbp. The average N50 contig length (L50) was 66,810 and the average GC content was 41.3%. Short read data are available on the NCBI SRA, associated with BioProject PRJNA418954. Assembled genomes and supplementary material are available from FigShare (10.6084/m9.figshare.13385465; individual accession numbers and assembled genome statistics in **Table S2**).

Population structure and phylogeny

A multisequence alignment was created from concatenated gene sequences of all core genes (found in >95% isolates) from the reference genome, BM407 (Holden et al., 2009) using MAFFT (Katoh et al., 2002) on a gene-by-gene basis (Morley et al., 2019)(size: 1,202,840 bp; **Supplementary file S1**). Maximum-likelihood phylogenies were reconstructed with IQ-TREE (version 1.6.8) using the GTR+F+I+G4 substitution model and ultra-fast bootstrapping (1,000 bootstraps) (Nguyen et al., 2015); and visualized on Microreact (Argimón et al., 2016): <https://microreact.org/project/Ssuis-ns2>

Accessory genome characterization

All unique genes present in at least one isolate (the pangenome) were identified by automated annotation using PROKKA (version 1.13) followed by PIRATE, a pangenomics tool that allows for orthologue gene clustering in bacteria (Seemann, 2014; Bayliss et al., 2019). We defined genes in

PIRATE using a wide range of amino acid percentage sequence identity thresholds for Markov Cluster algorithm (MCL) clustering (45, 50, 60, 70, 80, 90, 95, 98). The pan-genome of all 36 isolates contained 5,004 genes, of which 1,348 genes were shared by all isolates (>95%) and defined the core genome (**Table S3**). Pairwise core and accessory genome distances were compared using PopPUNK (version 1.1.4) (Lees et al., 2019), which uses pairwise nucleotide kmer comparisons to distinguish shared sequence and gene content to identify accessory genome divergence in relation to the core genome. A two-component Gaussian mixture model was used to build a network to define clusters (Components: 41; Density: 0.0579; Transitivity: 0.9518; Score: 0.8967).

Identification of virulence-associated genes

The accessory genome of each isolate was characterized, including detection of antimicrobial resistance genes, putative virulence factors, and known plasmid genes using ABRICATE (version 0.9.8) (<https://github.com/tseemann/abricate>) and the NCBI, VfDB, and PlasmidFinder databases (10th September 2019 update; **Tables S4, S5 and S6**) (Carattoli et al., 2014; Coordinators, 2018; Liu et al., 2019; Bortolaia et al., 2020). ABRICATE was used to identify antimicrobial resistance genes in the sequenced genomes by comparison with the NCBI database of 1,726 resistance genes covering 15 antimicrobial agent types; including genes associated with resistance to aminoglycosides, β -lactam, colistin, fluoroquinolone, fosfomycin, fusidic acid, glycopeptide, MLS-macrolide-lincosamide-streptogramin B, nitroimidazole, oxazolidinones, phenicols, rifampicin, sulphonamides, tetracycline, and trimethoprim. An astringent threshold of 98% identity was used for reporting a match between a gene in the NCBI database and the input genome. T-test and Fisher's exact test assessed statistical significance at 5% significance.

448 **Declarations**

449 **Ethical approval**

450 This study was carried out according to the guidelines for the care and use of laboratory animals
451 (National Research Council, 2010). The study protocol was approved by the Faculty of Veterinary
452 Medicine's Animal Care and Use Committee (Protocol number S24/2559).

453

454 **Data availability**

455 Short read data are available on the NCBI SRA, associated with BioProject PRJNA418954
456 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA418954>). Assembled genomes and supplementary
457 material are available from FigShare: doi: 10.6084/m9.figshare.13385465.

458

459 **Competing interests**

460 The authors declare no competing interests.

461

462 **Funding**

463 This research work was partially supported by Chiang Mai University and National research of
464 Thailand (HS2349). All high-performance computing was performed in collaboration with MRC
465 CLIMB, funded by the Medical Research Council (MR/L015080/1 & MR/T030062/1). Collaborative
466 visits between UK and Thai partners were supported by Newton Fund Researcher Links travel grant.
467 The funders played no role in study design or implementation.

468

469 **Author contributions**

470 NK: acquisition, analysis, and interpretation of data; drafted manuscript.

471 JKC: analysis, and interpretation of data; revised manuscript.

472 EM: analysis, and interpretation of data; revised manuscript.

473 MDH: acquisition and interpretation of data; revised manuscript.

474 SM: acquisition and interpretation of data; revised manuscript.

475 PakT: acquisition and interpretation of data; revised manuscript.

476 PacT: acquisition interpretation of data; revised manuscript.

KD: acquisition and interpretation of data; revised manuscript.

GM: interpretation of data; revised manuscript.

SKS: interpretation of data; revised manuscript.

PP: conceptualized and designed work; acquisition, analysis, and interpretation of data; drafted manuscript.

BP: conceptualized and designed work; acquisition, analysis, and interpretation of data; drafted manuscript.

Acknowledgments

We are grateful to all the technicians, scientists, and non-technical staff of the Veterinary Research and Development center (Upper northern region), Lampang, Thailand, and Swansea University medical school, Swansea, UK, for their cooperation and support.

Tables and Figures

Table 1. Antimicrobial susceptibility test results by disk diffusion method of 36 *S. suis*, grouped by serotype. Susceptible (S), intermediate (I) and resistant (R) phenotypes are indicated. Asterisk (*) indicates statistical significance by Pearson's Chi-square test and Fisher's exact test, p -value < 0.05.

Table 2. Antimicrobial resistance gene patterns of 36 *S. suis* isolates.

Table 3. Concordance of antimicrobial resistance phenotype and genotypes. Presence of resistance genes (G+) and number of phenotypically non-susceptible isolates (P+) indicated. Asterisk indicates p -value < 0.05 by Pearson's chi-square test, and Yate's correction for continuity.

Figure 1.

A: Isolates were collected as part of a survey of healthy pigs in Chiang Mai province, Thailand. **B:** Population structure of selected sequenced isolates compared with other serotype 2 genomes from the same region. All core genes (present in $\geq 95\%$ of isolates) from the reference genome (1,348 genes) were used to build a gene-by-gene alignment ($n = 36$; 1,202,840 bp). A maximum-likelihood

phylogeny was constructed with IQ-TREE, using a GTR model and ultrafast bootstrapping (1,000 bootstraps; version 1.6.8; Nguyen et al; 2015; Hoang et al 2018). Scale bar represents genetic distance of 0.01. Leaves are colored by disease status and host: samples from healthy pigs are green; diseased pigs are yellow; and samples from human clinical cases are red. Serotype 2 isolates are shaded in blue, with common STs annotated. The presence of antimicrobial resistance genes, known plasmids and virulence genes identified using ABRICATE and NCBI, PlasmidFinder and Vfdb databases are indicated by coloured blocks. Interactive visualization is available on Microreact: <https://microreact.org/project/Ssuis-ns2> (Argimon *et al*; 2016).

Figure 2.

A: Heatmap of pairwise average nucleotide identity (ANI). Highly similar pairwise comparisons are colored in red to blue for the most dissimilar isolates. The cluster of serotype 2 isolates are boxed. **B:** Summary of pairwise comparisons between serotype 2 and non-serotype 2 isolates. **C:** PopPUNK pairwise accessory distances visualized with t-SNE clustering in microreact: <https://microreact.org/project/Ssuis-ns2> (Argimon *et al*; 2016). **D:** Visualisation of the pangenome (PIRATE) with phandango, including estimation of the core (gene present in 95% or more isolates) and accessory genome composition (Bayliss et al; 2019; Hadfield et al 2018).

Figure 3.

A: Distribution of antimicrobial resistance patterns (black blocks indicate antimicrobial resistance genes identified in the genomes) summarized alongside their corresponding phenotypic resistance outcomes (red blocks indicated phenotypic resistance). Predicted (blue) and phenotypic (red) MDR is also indicated. **B:** Summary of the number of different antimicrobial classes to which each isolate demonstrated phenotypic resistance. Isolates resistant to three or more different antimicrobial classes were characterized as MDR.

Supplementary material

Table S1: Primers used for species and serotype identification.

534 **Table S2:** Summary of isolate genome statistics

535 **Table S3:** Summary of core and accessory genome characterization with PIRATE

536 **Table S4:** Summary of virulence genes identified by comparison with the VfDB database.

537 **Table S5:** Summary of AMR genes identified by comparison with the NCBI database.

538 **Table S6:** Summary of plasmid genes identified by comparison with the PlasmidFinder database.

539 **Table S7:** Matrix of gene presence for all plasmids identified by MOB-suite.

540

541 **Figure S1:** The effect of each ARG on phenotypic resistance diffusion diameters for
542 aminoglycosides, lincosamides, tetracyclines, phenicols, oxazolidinone and macrolide.

543

544 **Supplementary file 1:** Alignment file.

545

546 ***List of abbreviations***

547	AMC	Amoxycillin-Clavulanic acid
548	AMP	Ampicillin
549	AMR	Antimicrobial Resistance
550	AMX	Amoxycillin
551	ARGs	Antimicrobial Resistance Genes
552	CARD	Comprehensive Antibiotic Resistance Database
553	CHL	Chloramphenical
554	CLI	Clindamycin
555	CTF	Ceftiofur
556	DOX	Doxycycline
557	ENR	Enrofloxacin
558	ERY	Erythromycin
559	FLO	Florfenicol
560	GEN	Gentamycin
561	ICE	Integrative and Conjugative Elements
562	KAN	Kanamycin
563	LIN	Lincomycin
564	LZD	Linezolid
565	MDR	Multiple Drug Resistance
566	MLS	Macrolide-Lincosamide-Streptogramin B
567	NCBI	National Center for Biotechnology Information
568	OTC	Oxytetracyclin
569	PEN	Penicillin G
570	SXT	Sulfamethoxazole/Trimethoprim
571	TET	Tetracycline
572	VFDB	Virulence Factor Database

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A

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Chiang Mai province survey

Healthy pigs
(n=25)

Pig farms: 111 (25 districts)

Pigs sampled: 760

PCR positive *S. suis*: 138

Prevalence: 18.2%

Sequenced: 25

Comparison collection

Healthy pigs
(n=7)

Pig disease
(n=2)

Clinical disease
(n=2)

B

● Healthy pig ● Pig disease ● Human disease

A

Antimicrobial resistance gene patterns	Frequency	ant(6)-Ia aph(3')-IIIa aadE spw	Isa(E) Inu(B) Inu(C)	erm(A) erm(B) erm(T) mef(A) msr(D)	sat4	optrA	catA8	tet(O) tet(W) tet(L)	Predicted MDR	Gentamycin	Kanamycin	Lincomycin	Clindamycin	Erythromycin	Tetracycline	Doxycycline	Oxytetracycline	Linezolid	Chloramphenicol	Florfenicol	Ampicillin	Penicillin G	Amoxycillin	AMC	Enrofloxacin	Sulfa-tri	Ceftiofur	Phenotypic MDR
aadE/erm(B)/tet(O)	3 (8.3%)																											
ant(6)-Ia /erm(B)/mef(A)/msr(D)/tet(W)	3 (8.3%)																											
ant(6)-Ia /mef(A)/msr(D)/tet(W)	2 (5.6%)																											
ant(6)-Ia /spw/erm(B)/tet(O)	1 (2.8%)																											
ant(6)-Ia/aadE/spw/Isa(E)/Inu(B)/erm(B)/erm(T)/mef(A)/tet(O)/tet(L)	1 (2.8%)																											
ant(6)-Ia/aph(3')-III/erm(B)/sat4/tet(O)	2 (5.6%)																											
aph(3')-III/ant(6)-Ia /Isa(E)/Inu(B)/erm(B)	2 (5.6%)																											
ant(6)-Ia/erm(B)/tet(O)	1 (2.8%)																											
ant(6)-Ia/Inu(C)/erm(B)/mef(A)/msr(D)/tet(O)	1 (2.8%)																											
ant(6)-Ia/spw/Isa(E)/Inu(B)/erm(B)/tet(O)	1 (2.8%)																											
ant(6)-Ia/spw/Isa(E)/Inu(B)/erm(B)/tet(O)/tet(L)	1 (2.8%)																											
aph(3')-III/erm(B)/sat4/tet(O)	1 (2.8%)																											
erm(A)/erm(B)/optrA/catA8/tet(O)	1 (2.8%)																											
erm(A)/erm(B)/optrA/tet(O)	1 (2.8%)																											
erm(B)/erm(T)/tet(W)	1 (2.8%)																											
erm(B)/tet(O)	5 (13.9%)																											
spw	1 (2.8%)																											
spw/erm(B)/tet(O)	1 (2.8%)																											
spw/Isa(E)/Inu(B)/erm(B)/tet(O)	1 (2.8%)																											
tet(O)	2 (5.6%)																											
No resistance genes found	4 (11.1%)																											

B

Table 1. Antimicrobial susceptibility test results by disk diffusion method of 36 *S. suis*, grouped by serotype. Susceptible (S), intermediate (I) and resistant (R) phenotypes are indicated. Asterisk (*) indicates statistical significance by Pearson's Chi-square test and Fisher's exact test, p-value < 0.05

Antibiotics agents	Zone of inhibition (mm)			<i>S. suis</i> (%) (n=36)			Serotype 2 (%) (n=8)			Non-serotype 2 (%) (n=28)			p-value
	S	I	R	S	I	R	S	I	R	S	I	R	
GEN ^a	≥ 16	13-15	≤ 12	30.6	38.9	30.6	0	37.5	62.5	39.3	39.3	24.43	0.037*
KAN ^b	≥ 18	14-17	≤ 13	0	11.1	88.9	0	25	75	0	7.1	92.9	1.000
LIN ^c	≥ 19	16-18	≤ 15	0	0	100	0	0	100	0	0	100	1.000
CLI ^d	≥ 19	16-18	≤ 15	2.8	0	97.2	12.5	0	87.5	0	0	100	0.222
ERY ^d	≥ 21	16-20	≤ 15	16.7	13.9	69.4	12.5	0	87.5	17.8	14.3	67.9	0.719
TET ^d	≥ 28	25-27	≤ 24	2.8	5.6	91.7	0	0	100	3.6	7.1	89.3	0.588
DOX ^d	≥ 28	25-27	≤ 24	0	8.3	91.7	0	0	100	0	10.7	89.3	1.000
OTC ^c	≥ 26	16-25	≤ 15	5.6	11.1	83.3	0	0	100	7.1	14.3	78.6	1.000
LZD ^d	≥ 21	-	-	100	0	0	100	0	0	100	0	0	1.000
CHL ^d	≥ 21	18-20	≤ 17	44.4	47.2	8.3	0	100	0	57.1	32.1	10.7	0.010*
FLO ^e	≥ 22	19-21	≤ 18	72.2	22.2	5.6	75	25	0	71.4	21.4	7.1	0.689
AMP ^a	≥ 24	23-17	≤ 16	80.6	13.9	5.6	100	0	0	75	17.9	7.1	0.309
PEN ^b	≥ 26	13-25	≤ 12	30.6	66.7	2.8	87.5	12.5	0	14.3	82.1	3.6	0.001*
AMX ^f	≥ 24	15-23	≤ 14	83.3	13.9	2.8	100	0	0	78.6	17.9	3.6	0.299
AMC ^g	≥ 18	14-17	≤ 13	97.2	2.8	0	100	0	0	92.4	3.6	0	0.323
CTF ^e	≥ 21	18-20	≤ 17	94.4	5.6	0	100	0	0	92.9	7.1	0	1.000
ENR ^b	≥ 23	19-22	≤ 18	58.3	30.6	11.1	62.5	37.5	0	57.1	28.6	14.3	1.000
SXT ^d	≥ 19	16-18	≤ 15	72.2	5.6	22.2	87.5	0	12.5	67.9	7.14	25	0.160

+Antimicrobials used: GEN, gentamycin; KAN, kanamycin; LIN, lincomycin; CLI, clindamycin; ERY, erythromycin; TET, tetracycline; DOX, doxycycline; OTC, oxytetracycline; LZD, linezolid; CHL, chloramphenicol; FLO, florfenicol; AMP, ampicillin; PEN, penicillin G; AMX, amoxycillin; AMC, amoxycillin-clavulanic acid; CTF, ceftiofur; ENR, enrofloxacin; and SXT,
 ++Interpretative criteria according to: ^aCLSI 2017; ^bNEO-SENSITABS™ 2013; ^cCLSI 2008; ^dCLSI 2020;

Table 2. Antimicrobial resistance gene patterns of 36 *S. suis* isolates

Pattern	Antimicrobial resistance gene patterns	Strains	Frequency
A	<i>spw</i>	FH57	1 (2.8%)
B	<i>tet(O)</i>	DP-T2NE ^{†, β} , DP-T2N ^{†, β}	2 (5.6%)
C	<i>erm(B)/tet(O)</i>	H219-53 ^{α, β} , TSK11-2 ^β , TSK16-2 ^β , TP12-2 ^β , TP12-3 ^β	5 (13.9%)
D	<i>aadE/erm(B)/tet(O)</i>	FH11, FH12, FH52	3 (8.3%)
E	<i>ant(6)-Ia/erm(B)/tet(O)</i>	THD10-6	1 (2.8%)
F	<i>erm(B)/erm(T)/tet(W)</i>	FH13	1 (2.8%)
G	<i>spw/erm(B)/tet(O)</i>	FH25	1 (2.8%)
H	<i>aph(3')-III/erm(B)/sat4/tet(O)</i>	FH20	1 (2.8%)
I	<i>ant(6)-Ia /spw/erm(B)/tet(O)</i>	FH51	1 (2.8%)
J	<i>ant(6)-Ia /mef(A)/msr(D)/tet(W)</i>	FH16, FH17	2 (5.6%)
K	<i>erm(A)/erm(B)/optrA/tet(O)</i>	TJ21-2S	1 (2.8%)
L	<i>aph(3')-III/ant(6)-Ia /erm(B)/sat4/tet(O)</i>	FH9, FH10	2 (5.6%)
M	<i>aph(3')-III/ant(6)-Ia /lsa(E)/lnu(B)/erm(B)</i>	FH1, FH2	2 (5.6%)
N	<i>ant(6)-Ia /erm(B)/mef(A)/msr(D)/tet(W)</i>	FH19, FH42, FH43	3 (8.3%)
O	<i>erm(A)/erm(B)/optrA/catA8/tet(O)</i>	FH33	1 (2.8%)
P	<i>spw/lsa(E)/lnu(B)/erm(B)/tet(O)</i>	H132-53 ^{α, β}	1 (2.8%)
Q	<i>ant(6)-Ia/lnu(C)/erm(B)/mef(A)/mrd(D)/tet(O)</i>	FH24	1 (2.8%)
R	<i>ant(6)-Ia/lsa(E)/lnu(B)/erm(B)/tet(O)/tet(L)</i>	FH15*	1 (2.8%)
S	<i>ant(6)-Ia/spw/lsa(E)/lnu(B)/erm(B)/tet(O)</i>	FH50	1 (2.8%)
T	<i>ant(6)-Ia/aadE/spw/lsa(E)/lnu(B)/erm(B)/erm(T)/mef(A)/tet(O)/tet(L)</i>	T13-1N	1 (2.8%)
U	No resistance genes found	FH4-1, FH4-2, FH4-3, FH56	4 (11.1%)
Total			36 (100%)

* = *S. suis* serotype 9, ^α = *S. suis* from the human case, ^β = *S. suis* serotype 2, [†] = *S. suis* from diseased pigs

Table 3. Concordance of antimicrobial resistance phenotype and genotypes. Presence of resistance genes (G+) and number of phenotypically non-susceptible isolates (P+) indicated. Asterisk indicates p-value < 0.05 by Pearson's chi-square test, and Yate's correction for continuity.

Antimicrobial agents	AMR genes	Characterization of phenotypic and genotypic resistance (n=36)				Concordance of phenotypic and genotypic resistance	
		P+/G+	P-/G+	P+/G-	P-/G-	OR (95% CI)	p-value
GEN	<i>ant(6)-Ia</i>	8 (22.2%)	7 (19.4%)	16 (44.4%)	5 (13.9%)	0.36 (0.08- 1.42)	0.175
	<i>aph(3')-III</i>	-	5 (13.89%)	24 (66.7%)	7 (17.9%)	0.06 (0.005-0.46)	0.02
	<i>aadE</i>	3 (8.3%)	1 (2.8%)	21 (58.3%)	11 (30.6%)	1.57 (0.21-22.1)	0.851
	<i>spw</i>	3 (8.3%)	3 (8.3%)	21 (58.3%)	9 (25%)	0.43 (0.09-2.17)	0.635
KAN	<i>ant(6)-Ia</i>	15 (41.7%)	-	21 (58.3%)	-	0.71 (0.04-14.4)	0.615
	<i>aph(3')-III</i>	5 (13.9%)	-	31 (86.1%)	-	0.16 (0.01-3.64)	0.714
	<i>aadE</i>	4 (11.1%)	-	32 (88.9%)	-	0.13 (0.01-2.94)	0.611
	<i>spw</i>	6 (16.7%)	-	30 (83.3%)	-	0.2 (0.01-4.4)	0.805
LIN, CLI	<i>lsa(E)</i>	6 (16.7%)	-	30 (83.3%)	-	0.2 (0.01-4.4)	0.805
	<i>lnu(B)</i>	6 (16.7%)	-	30 (83.3%)	-	0.2 (0.01-4.4)	0.805
	<i>lnu(C)</i>	1 (2.8%)	-	35 (97.2%)	-	0.03 (0.002-1.06)	0.199
ERY	<i>erm(A)</i>	2 (5.6%)	-	28 (77.8%)	6 (16.7%)	0.43 (0.04-7.16)	0.745
	<i>erm(B)</i>	26 (72.2%)	1 (2.8%)	4 (11.1%)	5 (13.9%)	32.5 (3.79-390)	0.002*
	<i>erm(T)</i>	2 (5.6%)	-	28 (77.8%)	6 (16.7%)	0.43 (0.04-7.16)	0.917
	<i>mef(A)</i>	6 (16.7%)	1 (2.8%)	24 (66.7%)	5 (13.9%)	1.25 (0.16-16.9)	0.706
	<i>msr(D)</i>	5 (13.9%)	1 (2.8%)	25 (69.4%)	5 (13.9%)	1 (0.12-13.9)	0.548
TET	<i>tet(O)</i>	23 (63.9%)	-	12 (33.3%)	1 (2.3%)	1.92 (0.09-37.8)	0.758
	<i>tet(W)</i>	6 (16.7%)	-	29 (80.6%)	1 (2.8%)	0.207 (0.01-4.55)	0.821
	<i>tet(L)</i>	2 (5.6%)	-	33 (91.7%)	1 (2.8%)	0.06 (0.003-1.7)	0.368
DOX	<i>tet(O)</i>	23 (63.9%)	-	13 (36.1%)	-	1.77 (0.09-34.9)	0.721
	<i>tet(W)</i>	6 (16.7%)	-	30 (83.3%)	-	0.2 (0.01-4.40)	0.805
	<i>tet(L)</i>	2 (5.6%)	-	3 (94.4%)	-	0.06 (0.03-1.65)	0.357
OTC	<i>tet(O)</i>	23 (63.9%)	-	11 (30.6%)	2 (5.6%)	4.18 (0.43-62.7)	0.573
	<i>tet(W)</i>	6 (16.7%)	-	28 (77.8%)	2 (5.6%)	0.43 (0.04-7.16)	0.917
	<i>tet(L)</i>	2 (5.6%)	-	32 (88.9%)	2 (5.6%)	0.12 (0.01-2.65)	0.571
LZD	<i>optrA</i>	-	2 (5.6%)	-	34 (94.4%)	17 (0.61-327)	0.357
CHL	<i>catA8</i>	1 (2.8%)	-	19 (52.8%)	16 (44.4%)	0.84 (0.04-16.9)	0.541
FLO	<i>catA8</i>	1 (2.8%)	-	9 (25%)	26 (72.2%)	2.89 (0.14-56.6)	0.947