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

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Significance statement

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

Summary

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

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

Background

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

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

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

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

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

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 (≥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*)-*la*, *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 *Streptococcsu* 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 *dfr* 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,*

323 *tetM, tetO,* and *tetW* have been reported in *S. suis* (Holden et al., 2009; Athey et al., 2016b), however
324 plasmid elements were found only in non-serotype 2 isolates in this study.
325
326 **Conclusion**
327 We collected isolates from 760 healthy pigs reared in the pork industry in Northern Thailand.
328 Through comparison of 36 whole genome sequences, we identified increased genetic diversity in
329 these non-serotype 2 carriage isolates, from which the more invasive and pathogenic serotype 2
330 isolates emerge. Corresponding diversity was also seen in the breadth and diversity of AMR
331 determinants which conferred increased phenotypic non-susceptibility. This genetically diverse
332 reservoir of *S. suis* pose a public health risk with the potential for transmission to more invasive
333 isolates, broadening their spectrum of antimicrobial resistance. Extensive phenotypic resistance is
334 observed to antimicrobials that are not typically used to treat this infection. This can be partly
335 explained by the co-occurrence of resistance genes on ICEs. However, little phenotypic resistance was
336 observed to β-lactams, which remain the prescribed antimicrobial for *S. suis* infection in Thailand.
337 Continued surveillance and more stringent control of antimicrobial usage within the pork industry will
338 be necessary to monitor a growing AMR threat in *S. Suis.*
339
340

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 ½/1/2/7/9/14 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^{o}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 S**2).

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

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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.

477 KD: acquisition and interpretation of data; revised manuscript.
478 GM: interpretation of data; revised manuscript.
479 SKS: interpretation of data; revised manuscript.
480 PP: conceptualized and designed work; acquisition, analysis, and interpretation of data; drafted
481 manuscript.
482 BP: conceptualized and designed work; acquisition, analysis, and interpretation of data; drafted
483 manuscript.
484

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489

490 **Tables and Figures**

491 **Table 1.** Antimicrobial susceptibility test results by disk diffusion method of 36 *S. suis*, grouped by
492 serotype. Susceptible (S), intermediate (I) and resistant (R) phenotypes are indicated. Asterisk (*)
493 indicates statistical significance by Pearson's Chi-square test and Fisher's exact test, p-value < 0.05.
494

495 **Table 2.** Antimicrobial resistance gene patterns of 36 *S. suis* isolates.
496

497 **Table 3.** Concordance of antimicrobial resistance phenotype and genotypes. Presence of resistance
498 genes (G+) and number of phenotypically non-susceptible isolates (P+) indicated. Asterisk indicates
499 p-value < 0.05 by Pearson's chi-square test, and Yate's correction for continuity.
500

501 **Figure 1.**

502 **A:** Isolates were collected as part of a survey of healthy pigs in Chiang Mai province, Thailand. **B**:
503 Population structure of selected sequenced isolates compared with other serotype 2 genomes from the
504 same region. All core genes (present in ≥95% of isolates) from the reference genome (1,348 genes)
505 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.

Table S2: Summary of isolate genome statistics

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

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

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

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

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

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

Supplementary file 1: Alignment file.

List of abbreviations

AMC	Amoxycillin-Clavulanic acid
AMP	Ampicillin
AMR	Antimicrobial Resistance
AMX	Amoxycillin
ARGs	Antimicrobial Resistance Genes
CARD	Comprehensive Antibiotic Resistance Database
CHL	Chloramphenical
CLI	Clindamycin
CTF	Ceftiofur
DOX	Doxycycline
ENR	Enrofloxacin
ERY	Erythromycin
FLO	Florfenicol
GEN	Gentamycin
ICE	Integrative and Conjugative Elements
KAN	Kanamycin
LIN	Lincomycin
LZD	Linezolid
MDR	Multiple Drug Resistance
MLS	Macrolide-Lincosamide-Streptogramin B
NCBI	National Center for Biotechnology Information
OTC	Oxytetracyclin
PEN	Penicillin G
SXT	Sulfamethoxazole/Trimethoprim
TET	Tetracycline
VFDB	Virulence Factor Database

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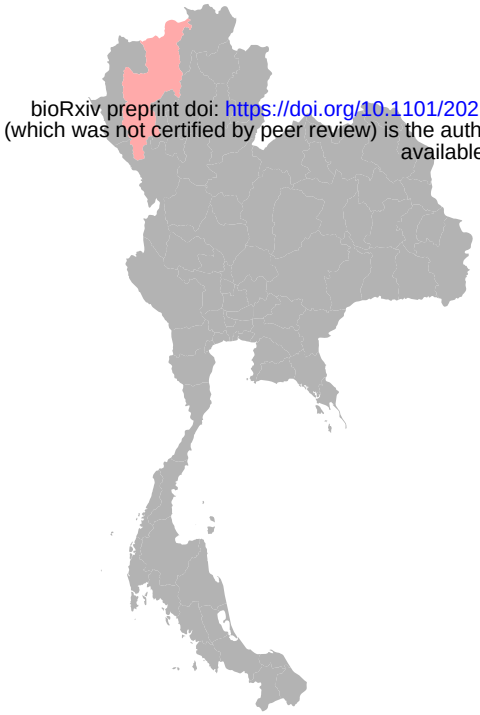
A



Chiang Mai province survey

From March - November 2015

Healthy pigs (n=25)

Pig farms: 111 (25 districts)
Pigs sampled: 760
PCR positive S. suis: 138
Prevalence: 18.2%
Sequenced: 25

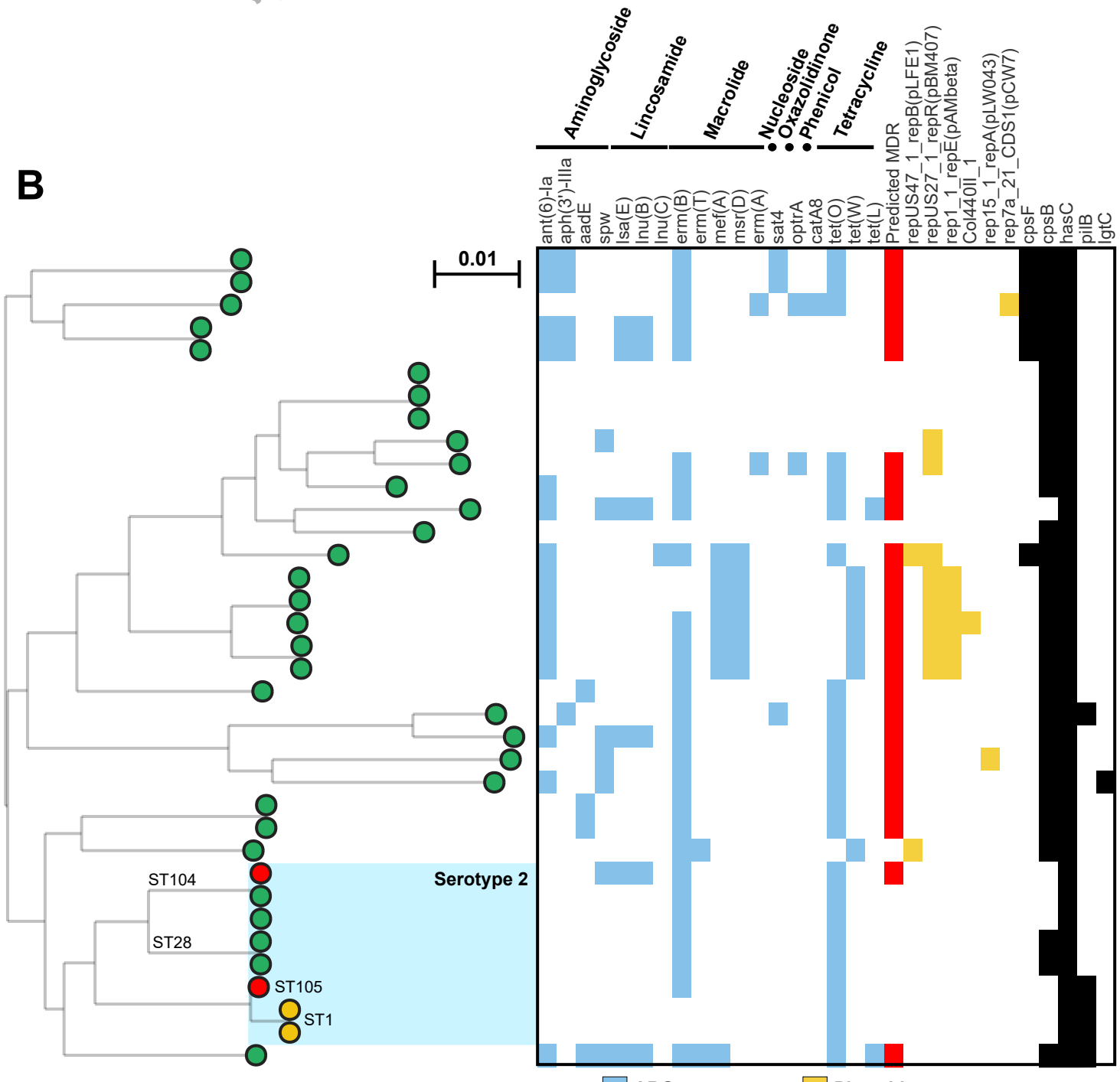
Comparison collection

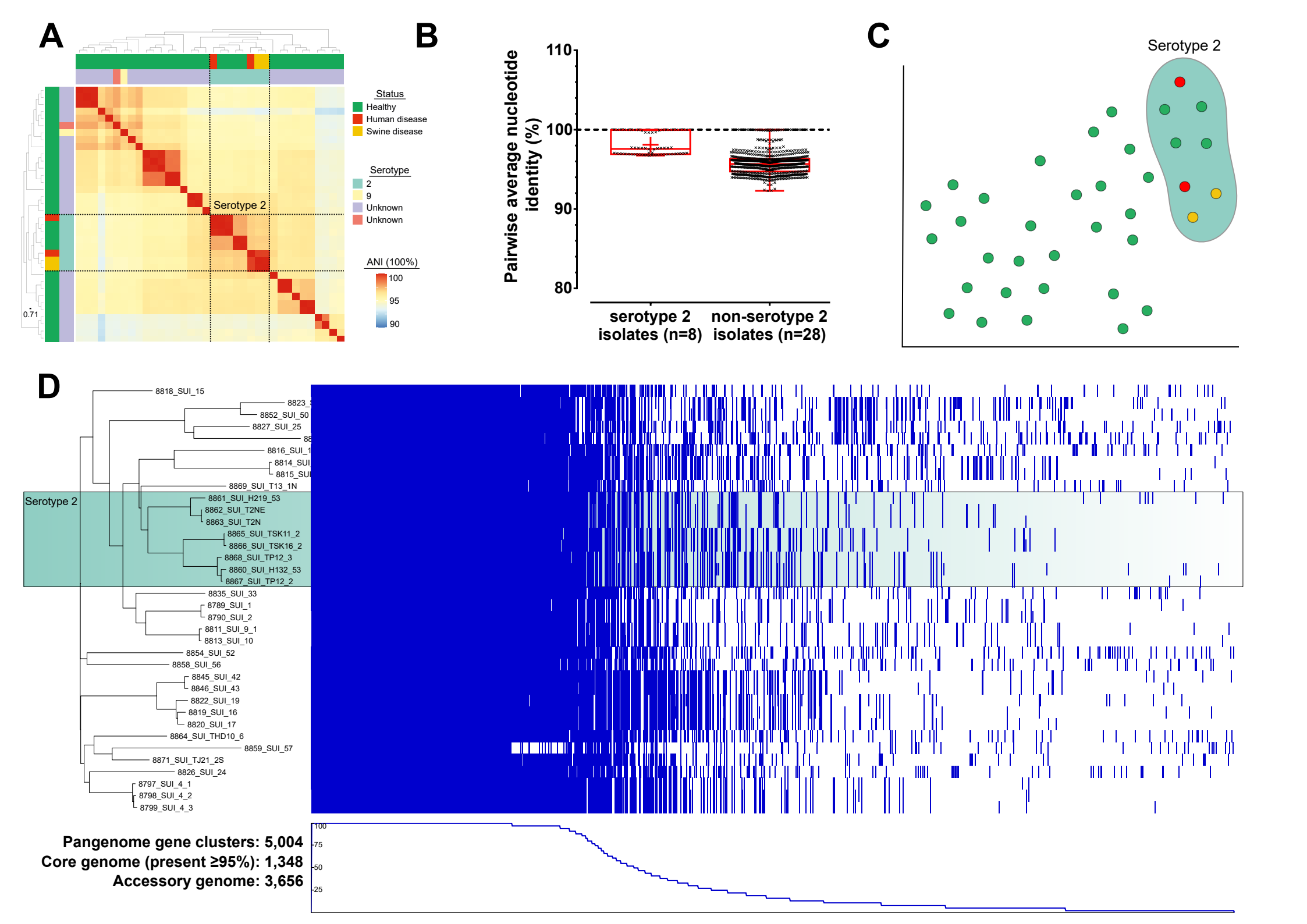
Healthy pigs (n=7)

Pig disease (n=2)

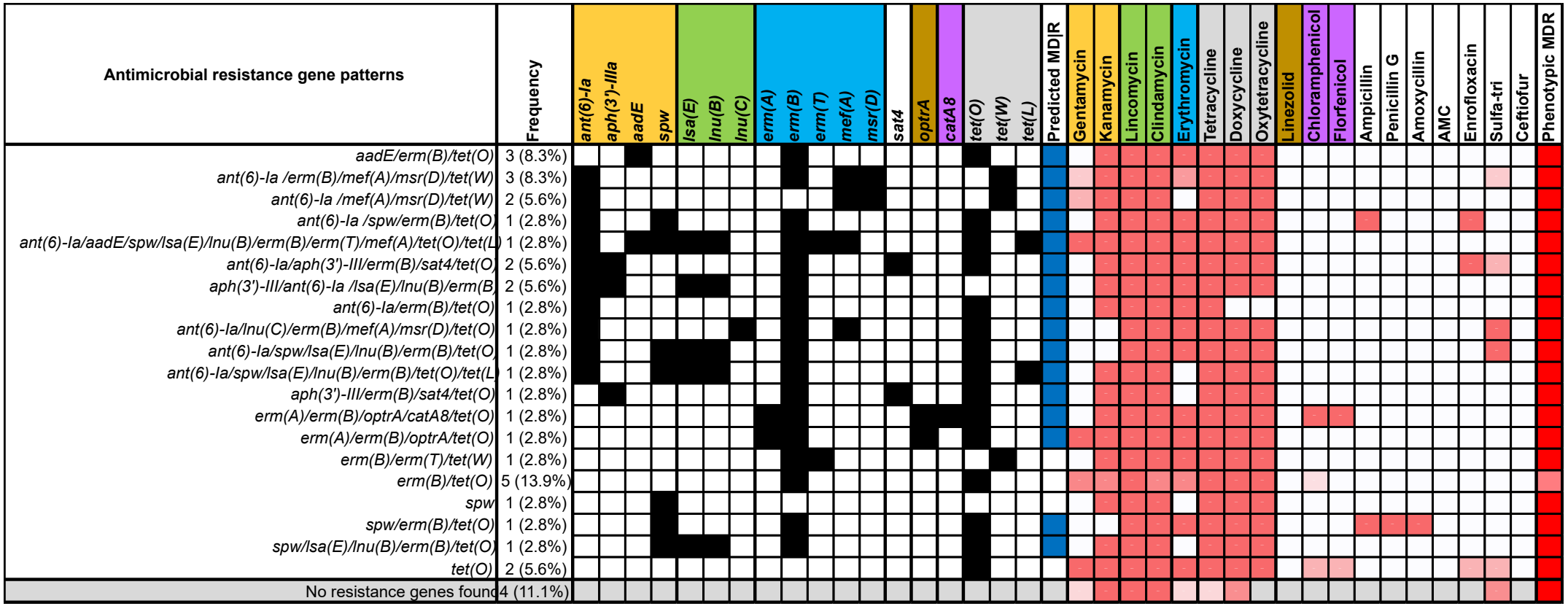
Clinical disease (n=2)

B




A
Status
Healthy
Human disease
Swine disease
Serotype
2
9
Unknown
Unknown
ANI (100%)
100
95
90
Serotype 2
0.71
B
Pairwise average nucleotide identity (%)
110
100
90
80
serotype 2 isolates (n=8)
non-serotype 2 isolates (n=28)
C
Serotype 2
D
8818_SUI_15
8823_
8852_SUI_50
8827_SUI_25
8816_SUI_1
8814_SUI
8815_SUI
8869_SUI_T13_1N
Serotype 2
8861_SUI_H219_53
8862_SUI_T2NE
8863_SUI_T2N
8865_SUI_TSK11_2
8866_SUI_TSK16_2
8868_SUI_TP12_3
8860_SUI_H132_53
8867_SUI_TP12_2
8835_SUI_33
8789_SUI_1
8790_SUI_2
8811_SUI_9_1
8813_SUI_10
8854_SUI_52
8858_SUI_56
8845_SUI_42
8846_SUI_43
8822_SUI_19
8819_SUI_16
8820_SUI_17
8864_SUI_THD10_6
8859_SUI_57
8871_SUI_TJ21_2S
8826_SUI_24
8797_SUI_4_1
8798_SUI_4_2
8799_SUI_4_3
100
75
50
25
Pangenome gene clusters: 5,004
Core genome (present ≥95%): 1,348
Accessory genome: 3,656

A


Antimicrobial resistance gene patterns
Frequency
ant(6)-Ia
aph(3')-IIIa
aadE
spw
lsa(E)
lnu(B)
lnu(C)
erm(A)
erm(B)
erm(T)
mef(A)
msr(D)
sat4
optrA
catA8
tet(O)
tet(W)
tet(L)
Predicted MDIR
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/lsa(E)/lnu(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 /lsa(E)/lnu(B)/erm(B) 2 (5.6%)
ant(6)-Ia/erm(B)/tet(O) 1 (2.8%)
ant(6)-Ia/lnu(C)/erm(B)/mef(A)/msr(D)/tet(O) 1 (2.8%)
ant(6)-Ia/spw/lsa(E)/lnu(B)/erm(B)/tet(O) 1 (2.8%)
ant(6)-Ia/spw/lsa(E)/lnu(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/lsa(E)/lnu(B)/erm(B)/tet(O) 1 (2.8%)
tet(O) 2 (5.6%)
No resistance genes found 4 (11.1%)

B

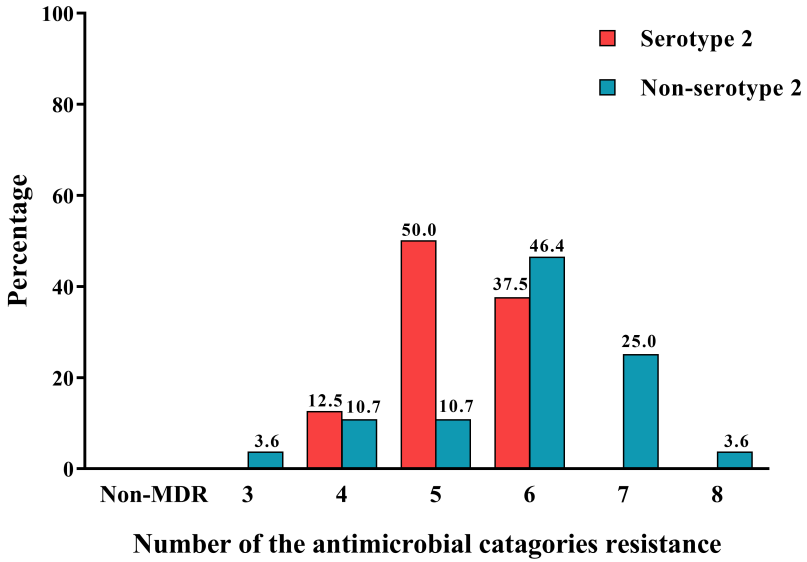

Serotype 2
Non-serotype 2
Percentage
100
80
60
40
20
0
3.6
12.5
10.7
50.0
10.7
37.5
46.4
25.0
3.6
Non-MDR
3
4
5
6
7
8
Number of the antimicrobial catagories resistance

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)			*S. suis* (%) (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: [a]CLSI 2017; [b]NEO-SENSITABS™ 2013; [c]CLSI 2008; [d]CLSI 2020;

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

Pattern	Antimicrobial resistance gene patterns	Strains	Frequency
A	*spw*	FH57	1 (2.8%)
B	*tet(O)*	DP-T2NE †, ꞵ, DP-T2N †, ꞵ	2 (5.6%)
C	*erm(B)/tet(O)*	H219-53α,ꞵ,TSK11-2ꞵ, TSK16-2ꞵ, TP12-2ꞵ, TP12-3ꞵ	5 (13.9%)
D	*aadE/erm(B)/tet(O)*	FH11, FH12, FH52	3 (8.3%)
E	*ant(6)-Ia/erm(B)/tet(O)*	THD10-6	1 (2.8%)
F	*erm(B)/erm(T)/tet(W)*	FH13	1 (2.8%)
G	*spw/erm(B)/tet(O)*	FH25	1 (2.8%)
H	*aph(3')-III/erm(B)/sat4/tet(O)*	FH20	1 (2.8%)
I	*ant(6)-Ia /spw/erm(B)/tet(O)*	FH51	1 (2.8%)
J	*ant(6)-Ia /mef(A)/msr(D)/tet(W)*	FH16, FH17	2 (5.6%)
K	*erm(A)/erm(B)/optrA/tet(O)*	TJ21-2S	1 (2.8%)
L	*aph(3')-III/ant(6)-Ia /erm(B)/sat4/tet(O)*	FH9, FH10	2 (5.6%)
M	*aph(3')-III/ant(6)-Ia /lsa(E)/lnu(B)/erm(B)*	FH1, FH2	2 (5.6%)
N	*ant(6)-Ia /erm(B)/mef(A)/msr(D)/tet(W)*	FH19, FH42, FH43	3 (8.3%)
O	*erm(A)/erm(B)/optrA/catA8/tet(O)*	FH33	1 (2.8%)
P	*spw/lsa(E)/lnu(B)/erm(B)/tet(O)*	H132-53 α, ꞵ	1 (2.8%)
Q	*ant(6)-Ia/lnu(C)/erm(B)/mef(A)/mrd(D)/tet(O)*	FH24	1 (2.8%)
R	*ant(6)-Ia/lsa(E)/lnu(B)/erm(B)/tet(O)/tet(L)*	FH15*	1 (2.8%)
S	*ant(6)-Ia/spw/lsa(E)/lnu(B)/erm(B)/tet(O)*	FH50	1 (2.8%)
T	*ant(6)-Ia/aadE/spw/lsa(E)/lnu(B)/erm(B) /erm(T)/mef(A)/tet(O)/tet(L)*	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, ꞵ = *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 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	*ant(6)-Ia*	8 (22.2%)	7 (19.4%)	16 (44.4%)	5 (13.9%)	0.36 (0.08- 1.42)	0.175
	aph(3')-III	-	5 (13.89%)	24 (66.7%)	7 (17.9%)	0.06 (0.005-0.46)	0.02
	aadE	3 (8.3%)	1 (2.8%)	21 (58.3%)	11 (30.6%)	1.57 (0.21-22.1)	0.851
	spw	3 (8.3%)	3 (8.3%)	21 (58.3%)	9 (25%)	0.43 (0.09-2.17)	0.635
KAN	*ant(6)-Ia*	15 (41.7%)	-	21 (58.3%)	-	0.71 (0.04-14.4)	0.615
	aph(3')-III	5 (13.9%)	-	31 (86.1%)	-	0.16 (0.01-3.64)	0.714
	aadE	4 (11.1%)	-	32 (88.9%)	-	0.13 (0.01-2.94)	0.611
	spw	6 (16.7%)	-	30 (83.3%)	-	0.2 (0.01-4.4)	0.805
LIN, CLI	*lsa(E)*	6 (16.7%)	-	30 (83.3%)	-	0.2 (0.01-4.4)	0.805
	lnu(B)	6 (16.7%)	-	30 (83.3%)	-	0.2 (0.01-4.4)	0.805
	lnu(C)	1 (2.8%)	-	35 (97.2%)	-	0.03 (0.002-1.06)	0.199
ERY	*erm(A)*	2 (5.6%)	-	28 (77.8%)	6 (16.7%)	0.43 (0.04-7.16)	0.745
	erm(B)	26 (72.2%)	1 (2.8%)	4 (11.1%)	5 (13.9%)	32.5 (3.79-390)	**0.002***
	erm(T)	2 (5.6%)	-	28 (77.8%)	6 (16.7%)	0.43 (0.04-7.16)	0.917
	mef(A)	6 (16.7%)	1 (2.8%)	24 (66.7%)	5 (13.9%)	1.25 (0.16-16.9)	0.706
	msr(D)	5 (13.9%)	1 (2.8%)	25 (69.4%)	5 (13.9%)	1 (0.12-13.9)	0.548
TET	*tet(O)*	23 (63.9%)	-	12 (33.3%)	1 (2.3%)	1.92 (0.09-37.8)	0.758
	tet(W)	6 (16.7%)	-	29 (80.6%)	1 (2.8%)	0.207 (0.01-4.55)	0.821
	tet(L)	2 (5.6%)	-	33 (91.7%)	1 (2.8%)	0.06 (0.003-1.7)	0.368
DOX	*tet(O)*	23 (63.9%)	-	13 (36.1%)	-	1.77 (0.09-34.9)	0.721
	tet(W)	6 (16.7%)	-	30 (83.3%)	-	0.2 (0.01-4.40)	0.805
	tet(L)	2 (5.6%)	-	3 (94.4%)	-	0.06 (0.03-1.65)	0.357
OTC	*tet(O)*	23 (63.9%)	-	11 (30.6%)	2 (5.6%)	4.18 (0.43-62.7)	0.573
	tet(W)	6 (16.7%)	-	28 (77.8%)	2 (5.6%)	0.43 (0.04-7.16)	0.917
	tet(L)	2 (5.6%)	-	32 (88.9%)	2 (5.6%)	0.12 (0.01-2.65)	0.571
LZD	*optrA*	-	2 (5.6%)	-	34 (94.4%)	17 (0.61-327)	0.357
CHL	*catA8*	1 (2.8%)	-	19 (52.8%)	16 (44.4%)	0.84 (0.04-16.9)	0.541
FLO	*catA8*	1 (2.8%)	-	9 (25%)	26 (72.2%)	2.89 (0.14-56.6)	0.947