

Preplanned Studies

Genomic Insight into the Antimicrobial Resistance of *Streptococcus Suis* — Six Countries, 2011–2019

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Summary

What is already known on this topic?

Streptococcus suis (*S. suis*) is a zoonotic pathogen causing disease in humans and animals, and the emergence of its increased resistance to antimicrobial agents has become a significant challenge in many countries.

What is added by this report?

Using whole genome sequencing data to accurately predict antimicrobial resistance determinants, it was found that the prevalence of antimicrobial resistance genes was higher in the pig isolates of *S. suis* than in the human isolates and that the prevalence of these genes varied with serotype.

What are the implications for public health practice?

The data regarding *S. suis* antimicrobial resistance will help guide rational drug use in the clinic to better protect the health of humans and animals.

Streptococcus suis (*S. suis*) is an important zoonotic pathogen that apart from causing huge economic losses to the global swine industry, can also cause severe illness and even death in humans. Antibiotics are commonly used to treat and control *S. suis* infections, contributing to the increasing prevalence of antimicrobial-resistant *S. suis*, which has attracted the attention of the global health community (1–3). Therefore, monitoring the prevalence of antimicrobial resistance-related genes in *S. suis* and studying its antimicrobial resistance characteristics will provide guidance for the clinical treatment of *S. suis* infections. In this study, *S. suis* isolates from 20 provincial-level administrative divisions (PLADs) in China were collected and analyzed from 2011 to 2019, together with the available *S. suis* sequencing data (with detailed host information) in the National Center for Biotechnology Information (NCBI) database, and a global surveillance of *S. suis* resistance was carried out according to several epidemiological dimensions. The results demonstrated that the prevalence of

antimicrobial resistance genes in *S. suis* was generally higher in China than in other countries and that the prevalence was generally higher in the pig isolates compared to the human isolates. The prevalence of resistance genes varied with serotype; however, *erm*(B), a gene mediating macrolide resistance, and *tet*(O), a gene mediating tetracycline resistance, were both prevalent across different serotypes. The results of drug sensitivity experiments indicated a high and concerning level of antimicrobial resistance in the *S. suis* isolated in China. Therefore, public health practitioners should pay attention to the rational use of medicines, reduce the abuse of therapeutic drugs, and promote the scientific use of medicines in farming to protect the lives of animals and humans.

In this study, nasal swabs, lung, spleen, liver, and lymph nodes of healthy and diseased pigs from 20 PLADs across China were collected and analyzed. The analysis data were then combined with the *S. suis* sequencing data (with detailed host information) in the NCBI database. The samples were collected strictly according to the relevant standard protocols, as all the samples were collected using Eswab tubes containing culture media and then stored and transported appropriately to the handling laboratories using ice packs. *S. suis* isolation was performed as described in previous studies (4). Genomic DNA of *S. suis* was extracted using the Wizard Genomic DNA Purification Kit (Promega, Beijing, China) following the manufacturer's instructions. DNA libraries were constructed using the KAPA HyperPrep Kit Illumina platforms (Roche, Basel, Switzerland) following standard protocols and then sequenced on an Illumina HiSeq X Ten platform (Annoroad, Beijing, China). Illumina sequencing reads for each isolate were processed using Trimmomatic with an average quality cutoff of 20 (2.3 million average reads per sample). Finally, the high-quality reads were assembled as contigs of at least 500 bp with SPAdes (with parameter-careful) (version 3.13.1, St. Petersburg

Academic University, <https://github.com/ablab/spades>). via the Unicycler (version 0.4.7, The University of Melbourne, <https://github.com/rrwick/Unicycler>) assembly pipeline (5). The quality of the genome sequences was checked using the default settings in QUAST (version 5.0.2, St. Petersburg Academic University, <https://github.com/ablab/quast>) (6). Identification of *S. suis* serotypes was performed using the protocol described by Athey et al. (7). Resistance genes were identified using the SRST2 Toolkit (version 0.2.0, The University of Melbourne, <http://katholt.github.io/srst2/>). Minimum inhibitory concentrations were determined by the broth microdilution method according to the recommendations of the Clinical and Laboratory Standards Institute guidelines (CLSI, 2015: M100-S25).

During 2011–2019, a total of 436 *S. suis* isolates were collected from 20 PLADs in China, including 271 isolates from healthy pigs and 165 from diseased pigs (Table 1). Upon analysis, all 436 isolates were identified as *S. suis*. Genomic analysis revealed 17–1,753 contigs per isolate, with genome sizes ranging from 1,949,027 to 2,642,630 bp and an average GC content of 41.12%. Additionally, 934 sequenced *S. suis* isolates with specific host sources were screened from 2,452 isolates in the NCBI database, including 461 isolates derived from humans, 129 from healthy pigs, and 344 from diseased pigs. Altogether, 1,370 *S. suis* isolates were obtained from China, Vietnam, Thailand, the Netherlands, the United Kingdom and Japan, and majority of them were obtained during 2011–2019. A total of 27 distinct serotypes were identified from the 1,370 isolates, with the predominant serotype being serotype 2 (n=686), which accounted for 50.1% of the isolates, followed by serotype 3 (n=75, 5.5%). Furthermore, 57 (4.2%), 54 (4.0%), 50 (3.6%), 32 (2.3%), 32 (2.3%), and 29 isolates (2.1%) were identified as belonging to serotype 9, 7, 4, 1, 8, and 14, respectively.

The predicted antimicrobial resistance genes using the SRST2 Toolkit (<http://katholt.github.io/srst2/>) in all 1,370 *S. suis* isolates indicated that the prevalence and number of antimicrobial resistance genes were diverse among the different serotypes (Figure 1). Among all the resistance genes, *erm*(B) and *tet*(O) were highly observed in all serotypes (Figure 1A). The newly characterized *srpA* gene, which confers resistance to streptogramin A, pleuromutilins, and lincosamides, was found in 60 isolates (4.4%), mainly in serotypes 2,

TABLE 1. Characteristics and prevalence of 436 *Streptococcus suis* from the 20 PLADs, China, 2011–2019.

PLADs	Host source (N)	
	Healthy pig	Diseased pig
Anhui	12	18
Beijing	27	0
Chongqing	28	0
Guangdong	57	0
Guizhou	1	0
Hebei	1	0
Heilongjiang	8	0
Henan	25	52
Hubei	18	22
Hunan	16	16
Inner Mongolia	1	0
Jiangsu	2	0
Jiangxi	2	19
Ningxia	1	0
Qinghai	2	0
shaanxi	0	18
Shandong	19	0
Shanxi	0	20
Sichuan	43	0
Xinjiang	8	0
Total	271	165

Note: Sequencing data obtained from the database were not shown.

Abbreviations: PLADs=provincial-level administrative divisions; N=number.

3, 4, 9, 12, 21, 25, 29, 30, and 31 (dominant serotype 2) (8). Further, the prevalence of antimicrobial resistance genes in the isolates from China was generally higher than that in the isolates from other countries. The prevalence of antimicrobial resistance genes in human isolates was lower than that in pig isolates (Figure 1C). Drug sensitivity results showed that the antimicrobial resistance rate of *S. suis* was the highest for macrolides (91.5%) and the lowest for β -lactams (3.9%–15.4%) (Table 2), consistent with the genomic analysis results of antimicrobial resistance.

DISCUSSION

In this study, the prevalence of antimicrobial resistance genes in *S. suis* was found to differ among the isolates from different sources; the prevalence of these genes in *S. suis* was generally higher in China than in foreign countries, which may be related to

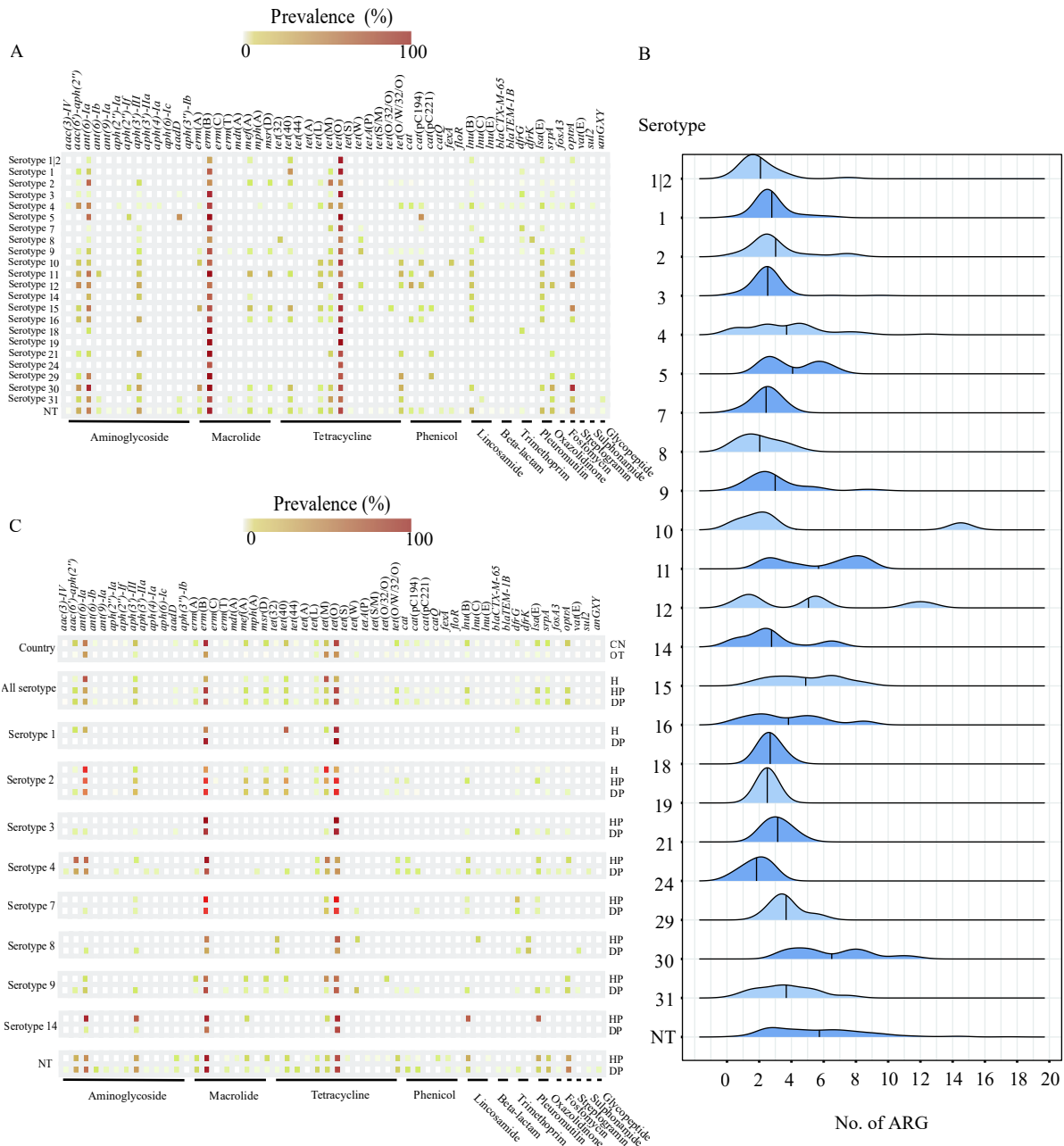


FIGURE 1. Prevalence and number of resistance genes among different serotypes in 1,370 *Streptococcus suis* isolates from 6 countries. (A) Frequencies of AMR genetic determinants within different serotypes, calculated across 22 serotypes. (B) Comparison of the number of AMR genes among the different serotypes. The median is represented by a black line. (C) Comparison of AMR gene prevalence among different countries or different hosts. The horizontal axis represents antimicrobial resistant genes and the vertical axis represents countries or host sources.

Note: In Figure 1A, cells indicate absence (white) or presence (colored by proportion) of each AMR determinant. The horizontal axis represents 54 antimicrobial resistance genes of 13 antibiotic classes, and the vertical axis represents 22 serotypes and none-type (NT), the number of which is greater than two. In Figure 1B, the median is represented by a black line. In Figure 1C, the horizontal axis represents antimicrobial resistant genes and the vertical axis represents countries or host sources.

Abbreviations: CN=China; OT=Other countries; H=Human; HP=Healthy pig; DP=Diseased pig; AMR=Antimicrobial resistance; ARG=Antibiotic resistance gene.

suboptimal drug use in breeding farms in China, indicating that the antimicrobial resistance of *S. suis* in China is a serious public health problem. With respect

to the host source, the prevalence of *S. suis* antimicrobial resistance genes in the human isolates was lower than that in the pig isolates. This may be

TABLE 2. The MIC distribution of 306 *Streptococcus suis* isolated in China (n=306).

Antimicrobials	MIC values ($\mu\text{g/mL}$)														Drug resistance rate (%)
	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1,024	
Aminoglycosides ^a	2	0	0	5	15	28	68	30	18	25	15	41	51	8	58.8%
Lincosamide ^b	9	0	0	0	69	0	0	1	9	7	12	75	106	18	74.5%
Fluoroquinolones ^c	100	25	24	73	2	24	16	23	13	6	0	0	0	0	27.5%
Tetracyclines ^d	0	6	0	2	9	45	32	33	25	39	43	5	1	0	58.2%
Macrolides ^e	2	4	20	0	3	19	22	21	19	17	51	111	17	0	91.5%
Chloramphenicol ^f	0	6	3	42	64	77	25	23	36	19	9	2	0	0	37.3%
Cephalosporins ^g	163	24	26	11	6	64	9	2	1	0	0	0	0	0	3.9%
Penicillins ^h	83	28	16	32	100	26	10	4	7	0	0	0	0	0	15.4%

Note: Refer to the streptococcal drug sensitive threshold.

Drug resistance threshold ($\mu\text{g/mL}$): a \geq 16, b \geq 4, c \geq 2, d \geq 8, e \geq 1, f \geq 8, g \geq 8, h \geq 4.

Abbreviation: MIC=minimum inhibitory concentration.

because most human isolates were serotype 2 and the prevalence of antimicrobial resistance genes in serotype 2 was generally lower than that in other serotypes, though the serotype 2 isolates accounted for a large proportion of the population. In general, the prevalence of *erm*(B), which mediates macrolide resistance, and *tet*(O), which mediates tetracycline resistance, was high among the isolates from all sources; however, the prevalence of these two genes in serotype 2 was lower than that in other serotypes, although serotype 2 represents the dominant serotype of pathogenic *S. suis*. Tetracycline resistance in porcine streptococci has become a major problem worldwide and is closely related to the widespread use of tetracyclines in the pig industry. The antimicrobial resistance gene prevalence and the antimicrobial resistance phenotype of *S. suis* evaluated in this study indicate the problem of the antimicrobial resistance of *S. suis* in China to be prominent, and timely actions should be taken to avert an imminent crisis. The results further provide important guidance and reference values for clinical drug use.

The findings of this study were consistent with those of some previous studies. Hout et al. showed that among the 1,163 isolates of *S. suis* collected in the Netherlands during 2013–2015, 78.4% were resistant to tetracycline and 48.1% to clindamycin, with the resistance rate for β -lactams being less than 5% (9). Another study by Zhang et al. investigating the drug sensitivity of 421 *S. suis* isolates from China found that the antimicrobial resistance rates for tetracycline, macrolides, and sulfonamides were more than 60%, and the antimicrobial resistance rate for β -lactams was only 9.5% (10). Based on these results, β -lactam antibiotics can still be used as the drugs of choice for *S. suis* infections.

This study was subject to some limitations. While the isolates used in this study for genomic analysis covered most parts of China; the geographical locations of the isolates whose data was sourced from GenBank were not evenly distributed. In addition, most of the isolates from Vietnam belonged to serotype 2.

In summary, data of 1,370 isolates of *S. suis* from 6 countries were investigated for antimicrobial resistance and classified according to different characteristics for comparative analysis. The prevalence of macrolide and tetracycline resistance genes was the highest. The prevalence of the resistance genes varied with the source; it was lower in the human isolates of *S. suis* than in the pig isolates. Based on these findings, to limit the extensive spread of antimicrobial-resistant *S. suis*, a series of interventional policies are needed to address the impending antimicrobial resistance crisis and to develop appropriate dosing strategies for the different strains of *S. suis* with different hosts to protect the health of humans and animals under the “One Health” approach.

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