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Research Article

Antimicrobial Susceptibility, Serotypes, and Genetic Diversity of Streptococcus suis in Diseased and Healthy Pigs in Southern China

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Abstract

In the study, the characteristics of 81 *Streptococcus* suis isolates recovered from healthy and diseased pigs in Southern China were analyzed. These isolates showed differential resistance rates of over 40% to 22 and over 80% to 11 antibiotics out of 42 tested drugs. These isolates belonged to 6 serotypes (2, 3, 7, 9, 19, and 28), 9.9% of which were characterised as non-typable (NT), and were classified into 15 sequence types (STs), 8 of which were new (ST812-ST815, ST821-823, and ST832). Additionally, 6 virulence-associated gene patterns (VAGPs) and 10 random amplified polymorphic DNA (RAPD) patterns were found among the 81 strains. Among the 4 genotyping methods used in this study, multilocus sequence typing (MLST) provided the highest resolution for identification of S. suis strains. Significant differences in STs, RAPD patterns, and VAGPs were observed between strains isolated from healthy pigs and those isolated from diseased pigs. This study did not find any statistically significant correlations between antimicrobial resistance and serotypes, STs, RAPD patterns, VAGPs, or strain sources. Serotype 2 strains were predominant (44.4%) in the study and all of these strains showed the molecular characteristics of highly pathogenic strains in VAGP, GDH sequence types, and MLST. Our results showed that *S. suis* strains in Southern China are continually evolving, and therefore increased surveillance of *S. suis* in piggeries must be considered.

Keywords: *Streptococcus suis, Antimicrobial susceptibility, Multilocus sequence typing, Virulence genes, RAPD, Glutamate dehydrogenase*

Güney Çin'de Hastalıklı ve Sağlıklı Domuzlarda *Streptococcus suis*'in Antimikrobiyal Direnci, Serotipleri ve Genetik Çeşitliliği

Öz

Bu çalışmada Güney Çin'de sağlıklı ve hasta domuzlardan elde edilen 81 *Streptococcus suis* izolatının özellikleri incelendi. İzolatlar, test edilen 42 antibiyotikten 22'sine %40'ın üzerinde ve 11'ine %80'in üzerinde değişen direnç gösterdi. Bu izolatlar 6 serotipe (2, 3, 7, 9, 19 ve 28) ait olup %9.9'u tiplendirilemeyen olarak karakterize edildi ve 8'i yeni (ST812-ST815, ST821-823 ve ST832) olmak üzere 15 sekans tipine (ST) sınıflandırıldı. Ayrıca, 81 suşta 6 adet virulans ile ilişkili gen şeması (VAGP) ve 10 adet rastgele amplifiye polimorfik DNA (RAPD) şeması bulundu. Çalışmada kullanılan 4 genotiplendirme metodu arasında multilokus sekans tiplendirme (MLST) S. suis suşlarının tespitinde en yüksek rezolusyonu sağladı. Sağlıklı ve hasta domuzlardan izole edilen suşlar arasında sekans tiplerinde, RAPD şemasında ve VAGP'lerde anlamlı farklılıklar gözlemlendi. Çalışmada antimikrobiyal direnç ile serotipler, ST, RAPD şeması, VAGP'ler veya suş kaynakları arasında istatistiksel olarak bir ilişki tespit edilmedi. Serotip 2 suşu çalışmadaki predominant olandı ve tüm bu suşlar VAGP, GDH sekans tipi ve MIST'de oldukça patojenik suşların moleküler özelliklerini göstermekteydi. Elde edilen sonuçlar Güney Çin'de *S. suis* suşlarının sürekli olarak değiştiğini ve bu yüzden domuzlarda *S.suis* takibinin gerekli olduğunu göstermiştir.

Anahtar sözcükler: *Streptococcus suis, Antimikrobiyal direnç, Multilokus sekans tiplendirmesi, Virulans genler, RAPD, Glutamat dehidrogenaz*

INTRODUCTION

Streptococcus suis (S. suis) is an opportunistic zoonotic pathogen. *S. suis* can infect humans and various animals, causing many serious diseases such as meningitis, septicemia, endocarditis, pneumonia, and pyogenic arthritis [1]. At least 33 serotypes (1-31, 1/2, and 33) of *S. suis* have been described according to the differences in capsule antigen [2-4]. In recent years, novel variant serotypes of *S. suis* have been reported [5].

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Serotyping of *S. suis* is one of the most useful methods to understand the pathogen epidemiology. Although the disease largely should be mostly caused by a small number of serotypes, it is recognized that the capsular serotype is a poor marker of virulence. Not all isolates allocated to the same serotype cause the same type of disease, and virulence can vary substantially both within and among serotypes [6]. Differences in virulence among *S. suis* isolates are related to virulence-associated genes. At present, more than 70 virulence-associated genes have been found in *S. suis*, most of which are bacterial surface components, surface proteins, extracellular proteins, enzymes, and regulatory factors. These virulence genes are directly or indirectly involved in adhesion to host cells, survival *in vivo,* and immune escape [7]. By analyzing the main virulence genes of *S. suis* strains, it would be easier to understand the molecular characteristics and pathogenicity of the strains [8-10].

Antimicrobial agents play an important role in the treatment and control of *S. suis* infection. However, irrational use of antibiotics leads to a continual increase in drug resistance of *S. suis* worldwide [11-15]. Therefore, antimicrobial susceptibility testing is a crucial step preceding the prescription of antibiotic treatment.

S. suis genotyping and associated research techniques can be used to compare the epidemic strains from different regions, better understand *S. suis* epidemiology, predict the origins, causes, and epidemic trends of the disease. *S. suis* genotyping has been carried out using a variety of methods [16-18].

In this study, the antimicrobial susceptibility of 81 isolates from healthy and diseased pigs in Southern China were tested against 42 antimicrobial agents. These isolates were characterized by serotyping, multilocus sequence typing (MLST), random amplified polymorphic DNA (RAPD), virulence-associated gene pattern (VAGP), and sequence analysis of the gene encoding glutamate dehydrogenase (GDH). The potential relationship between antimicrobial resistance and these characteristics was also analyzed.

MATERIAL and METHODS

Strains and Serotyping

A total of 81 *S. suis* strains were isolated from healthy and diseased pigs in Southern China between 2013 and 2016. Fourteen were from the nasal cavity of healthy pigs and 67 were from the blood, joint fluid, and lungs of diseased pigs. The isolates were serotyped using the method described in Okural et al.[19].

Antimicrobial Susceptibility

The antimicrobial susceptibility of the *S. suis* isolates was assessed using the disk diffusion method described by Soares et al.[20]. Antimicrobial disks (Hangzhou Tianhe Microbiological Co., Hangzhou, China) and associated concentration are described in *Table 1*. Growth inhibition was assessed against NCCLS standards and the isolates were classified as susceptible, intermediate or resistant. Strains ATCC 49619 *(Streptococcus pneumoniae)* was included as experimental control.

Detection of Virulence-Associated Genes

The virulence-associated genes extracellular protein factor *(epf),* murimidase-released protein *(mrp),* suilysin *(sly),* arginine deiminase *(arcA)*, 38-kDa protective antigen *(bay046),* hyaluronidase *(hyl),* and virulence-associated sequences *orf2* were detected by PCR, as previously described [21-25]. Details of all oligonucleotide primers used are listed in *Table 2*.

Nucleotide Sequence Determination of Glutamate Dehydrogenase

In this study, the *gdh* gene was PCR amplified using the primers GDHF (5'AACA TTCGGATTTTGCAATAAAAA3') and GDHR (5'ATTAGCACGTCAATTTTGGGG3'). The primers were designed using a previously reported *gdh* nucleotide sequence in GenBank (GenBank accession number AF229683) [26]. Subsequently, the PCR product obtained from each isolate was sub-cloned into the pUCm-T vector and sequenced using the dideoxy chain-termination method (Sangon Biotech Ltd., Shanghai, China). GDH sequence types of the *S. suis* isolates were analyzed using the method described by Kutz et al.[27].

DNA Fingerprinting by Random Amplified Polymorphic DNA

RAPD of the *S. suis* isolates was performed based on the method previously described [28-30]. The RAPD primer S298 (5'-GTGGAGTCAG-3') was used to analyze *S. suis* isolates. PCR reaction mixtures contained 10 mmol/L Tris-HCl (pH8.3), 10 mmol/L KCl, 2.0 mmol/L $MgCl_2$, 0.2 mmol/L dNTP, 1.5 U Taq DNA polymerase, 0.5 μmol/L of each primer, and 1.0 uL of genomic DNA. The PCR conditions were as follows: 1 cycle at 94°C for 2 min; 40 cycles at 94°C for 30 sec, 36°C for 30 sec, and 72°C for 2 min; and a cycle at 72°C for 6 min. The amplified products were analyzed using 1.4% agarose gel electrophoresis.

Multilocus Sequence Typing

A total of seven housekeeping genes were used in the MLST analysis of the *S. suis* isolates: *aroA*, *cpn60*, *dpr*, *gki*, *mutS*, *recA*, and *thrA*. These genes were amplified by PCR described in King et al.[31]. The PCR products were sequenced from both directions by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). The housekeeping gene sequences, obtained for each isolate, were edited, assembled, and aligned by the software MEGA version 5.0 [32]. Then, the sequences were submitted to the MLST

database *(https://pubmlst.org/ssuis/)* for the analysis of allele number and sequence type (ST) according to the methodology described by Jolley et al.[33]. The population structure and clonal complexes of the *S. suis* isolates were determined using the eBURST software [34].

RESULTS

In this study, the antibiotic resistance of the 81 *S. suis* isolates against 42 different antibiotics were tested. The resistance rates to 11 antibiotics were high (over 80%), to 11 antibiotics were intermediate (40-80%) and to 15 antibiotics were low (beow 30%) *(Table 1)*. Furthermore, all isolates showed 0% resistance to cefradine, cefazolin, teicoplanin, vancomycin and rifampicin *(Table 1)*.

Six distinct serotypes within the 81 serotyped *S. suis* isolates were identified. Serotype 2 (44.4%) was the most prevalent, followed by serotypes 7 (22.2%), 3 (8.6%), 9 (7.4%), 19 (4.9%), and 28 (2.5%). A total of 9.9% of the isolates tested were non-typable (NT). All isolates from diseased pigs were classified as serotypes 2, 3, 7, and 9. All of the isolates from healthy pigs were classified as serotypes 19, 28, and NT *(Table 3)*.

There were 6 kinds of VAGPs in all 81 strains tested. All *S. suis* serotype 2 strains were positive for 7 virulence genes and were classified as VAGP1. Serotype 3, 7, and 9 strains were positive for 5 virulence genes and were classified as VAGP2 (they lacked *epf* and *siy)*. There were 4 kinds of VAGPs in 14 strains from healthy pigs. All strains were positive for virulence genes *acrA* and *bay046*. Seventy-nine strains (97.5%) were positive for virulence genes *mrp*, while only 2 strains from healthy pigs were negative. Seventy-six strains (93.8%) were positive for virulence genes *orf2* and only 5 strains from healthy pigs were negative. All of the strains from diseased pigs were positive for virulence genes *hyl* and all of the strains from healthy pigs were negative for virulence genes *hyl*. Virulence genes *epf* and *siy* were only distributed in serotype 2 strains *(Table 3)*.

In the study, RAPD patterns of 3 to 8 bands with sizes ranging between 300 and 2.000 bp were obtained using RAPD primer S298. The 81 *S. suis* strains showed 10 RAPD patterns (RAPD-A to RAPD-J) according to the differences in numbers and band sizes *(Table 3)*.

In this study, we identified 2 new alleles *(cpn60* 311 and *cpn60* 312). A total of 15 STs were found and 8 STs (ST812-ST815, ST821-823, and ST832) were new MLST STs. ST1, ST86, ST242, ST253, ST812, and ST813 belonged to the clonal complex 1 (CC1), ST117 and ST29 belonged to CC2, ST243 and ST814 belonged to CC55, ST815 belonged to CC67, ST821 and ST822 belonged to CC71, ST823 belonged to CC53, and ST833 were singletons *(Table 3; Fig. 1)*.

Highly virulent strains of *S. suis* serotype 2 can be distinguished from moderately virulent and nonvirulent strains on the basis of their GDH [26,27]. The isolates of *S. suis* serotype 2 were divided into 3 groups according to deduced GDH amino acid sequence. Group I consisted of highly virulent isolates, containing Ala299-to-Ser, Glu305-to-Lys, and Glu330-to-Lys amino acid substitutions compared

Table 1. *Antimicrobial resistance profiles of S. suis strains from southern China*

Antibiotic Tested (μg)	Level of Susceptibility		
	Resistant (%)	Intermediate (%)	Sensitive (%)
Clarithromycin (15)	70.4	3.7	25.9
Azithromycin (15)	81.5	0	18.5
Erythromycin (15)	70.4	3.7	25.9
Roxithromycin(15)	74.1	3.7	22.2
Amoxicillin (10)	22.2	0	77.8
Ampicillin (10)	29.6	0	70.4
Carbenicillin (100)	40.7	0	59.3
Penicillin (10IU)	14.8	0	85.2
Cefradine (30)	0	0	100
Cefazolin (30)	0	0	100
Cefalexin (30)	3.7	3.7	92.6
Cefixime (30)	81.5	11.1	7.4
Cefotaxime (30)	22.2	0	77.8
Cefatriaxone (30)	11.1	0	88.9
Cefuroxime (30)	7.4	25.9	66.7
Gentamicin (10)	77.8	11.1	11.1
Kanamycin (30)	66.7	29.6	3.7
Amikacin (30)	88.9	3.7	7.4
Spectinomycin (100)	3.7	0	96.3
Streptomycin (10)	48.1	18.5	33.3
Tetracycline (30)	85.2	3.7	11.1
Deoxytetracycline (30)	22.2	40.7	37.1
Lomefloxacin (10)	85.2	11.1	3.7
Enrofloxacin (5)	3.7	0	96.3
Ciprofloxacin (5)	11.1	63	25.9
Norfloxacin (10)	44.4	29.6	25.9
Fleroxacin (10)	48.1	33.3	18.5
Levofloxacin (5)	3.7	3.7	92.6
Ofloxacin (5)	3.7	0	96.3
Enoxacin (10)	81.5	14.8	3.7
Nalidixan (30)	100	0	0
Trimethoprim (1.25) + Sulfamethoxazole (23.75)	44.4	7.4	48.1
Sulfisoxazole (300)	85.2	7.4	7.4
Trimethoprim	70.4	3.7	25.9
Clindamycin (30)	81.5	0	18.5
Cillimycin (2)	96.3	0	3.7
Chloramphenicol (30)	7.4	25.9	66.7
Teicoplanin (30)	0	3.7	96.3
Vancomycin (30)	0	0	100
Foroxone (300)	92.6	0	7.4
Furadantin (300)	22.2	29.6	48.1
Rifampicin (5)	0	7.4	92.6

Table 2. *Oligonucleotide primer sequences used in this study*

Genes	Primer Sequence (5'-3')	PCR Product Size (bp)	Reference
gdh	AACATTCGGATTTTGCAATAAAAA ATTAGCACGTCAATTTTGGGG	1500	This study
epf	CGCAGACAACGAAAGATTGA AAGAATGTCTTTGGCGATGG	744	[21]
sly	ACTCTATCACCTCATCCGC ATGAGAAAAAGTTCGCACTTG	1400	[22]
mrp	ATTGCTCCACAAGAGGATGG TGAGCTTTACCTCATCCGC	188	[21]
hyl	CTCAGATGAAAGCCTTTCTA TTTGTCCTTGGTCGTTGTC	1290	[23]
arcA	TGATATGGTTGCTGCTGGTC GGACTCGAGGATAGCATTGG	118	[21]
bay046	ATGCCACGGATTACCTTCCC CCGTCTCCTTAATGATCCGC	253	[24]
orf2	CAAGTGTATGTGGATGGG ATCCAGTTGACACGTGCA	858	[25]

Table 3. *Source of isolation and characteristics of the 81 S. suis isolates in this study*

ST/ CC	Presence of Virulence-Associated Genes							VAGP	Serotype	RAPD Pattern	Number of Strains	Source
	epf	sly	mrp	hyl	orf2	acrA	bay046					
1/1	+	+	+	+	+	+	+	1	2	A	19	The blood, joint fluid and lung of diseased pigs
86/1	+	+	+	+	+	+	+	1	2	A	2	
812	+	+	+	+	+	+	+	1	2	A	2	
813	+	+	+	+	+	+	+	1	2	A	2	
242	+	+	+	+	+	+	+	1	2	B	9	
253	+	+	+	+	+	+	+	1	2	C	2	
117/2	-	-	+	+	+	+	+	2	3	D	7	
29/2	-	-	+	+	+	+	+	2	7	E	18	
243/55	-	-	+	+	+	+	+	2	9	F	4	
814/55	-	-	+	+	+	+	+	2	9	F	2	
815/67	-	-	+	-	+	+	+	3	19	G	4	The nose swabs of healthy pigs
833/-	-	-	+	-	+	+	+	3	28	H	2	
821/71	-	-	-	-	+	+	+	4	NT	I	1	
821/71	-	-	+	-	-	+	+	5	NT	I	2	
821/71	-	-	-	-	-	+	+	6	NT	I	1	
822/71	-	-	+	-	+	+	+	3	NT	I	2	
823/53	-	-	+	-	-	+	+	5	NT	J	2	

with groups II and III. Groups II and III consisted of moderately virulent and nonvirulent strains, which are separated from each other by Tyr72-to-Asp and Thr296-to-Ala substitutions [27]. In this study, the GDH gene of 36 strains of *S. suis* serotype 2 obtained a 1.500-bp fragment containing an open reading frame of 1.344 nucleotides by PCR and nucleotide sequence determination. According to the deduced amino acid sequence, these strains were all classified as GDH Group I.

The resistance to 42 antimicrobial agents among each characteristic of all these isolates as a whole was counted and compared it with the distribution of serotypes, STs, RAPD patterns, VAGP, and strain sources to the distribution of antimicrobial resistance. The isolation frequency of each serotype was very similar to the incidence of antimicrobial resistance in the corresponding serotype, the isolation frequency of each ST was very similar to the incidence of antimicrobial resistance in the corresponding ST, the isolation frequency of each RAPD pattern was very similar to the incidence of antimicrobial resistance in the corresponding RAPD pattern, the isolation frequency of each VAGP was very similar to the incidence of antimicrobial resistance in the corresponding VAGP, and the isolation frequency of diseased pigs or healthy pigs was also very similar to the incidence of antimicrobial resistance in the corresponding source of strains. The statistical results showed that there was no significant correlation between antimicrobial resistance and serotypes, STS, RAPD patterns, or VAGPs *(Fig. 2-Fig. 6)*.

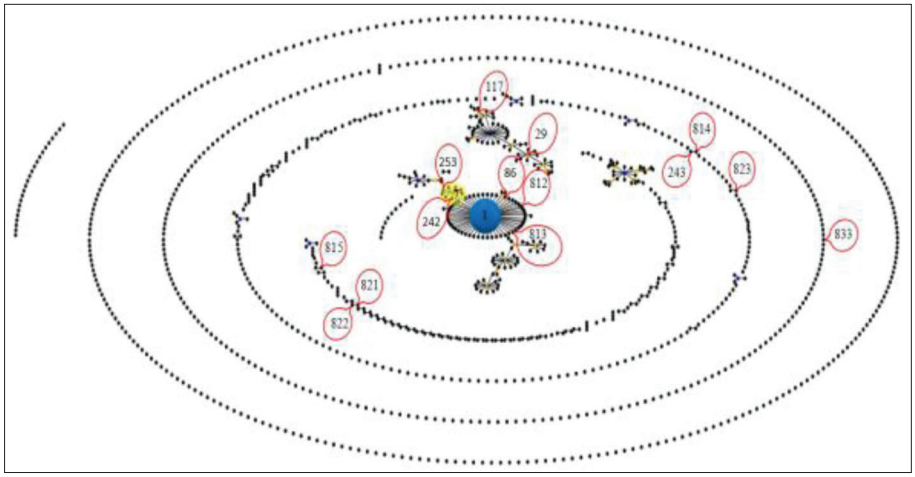
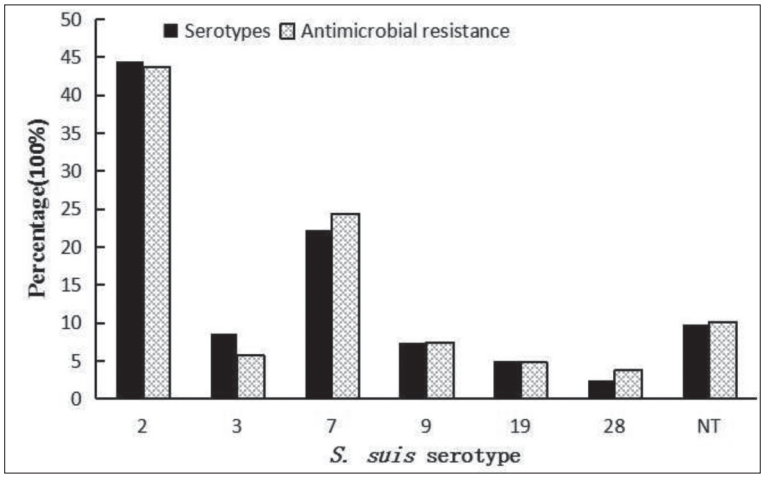


Fig 1. The population structure based 1096 STs from the MLST database of *Streptococcus suis*

Fig 2. Distribution of *S. suis* serotypes and antimicrobial resistance among each of these serotypes. Serotypes: frequency (%) = number of strains belonging to a certain serotyp/total number of strains ×100%; Antimicrobial resistance: incidence (%) = number of resistances to all tested antimicrobial agents among strains of a certain serotype/ total number of resistances to all tested antimicrobial agents across all strains×100%



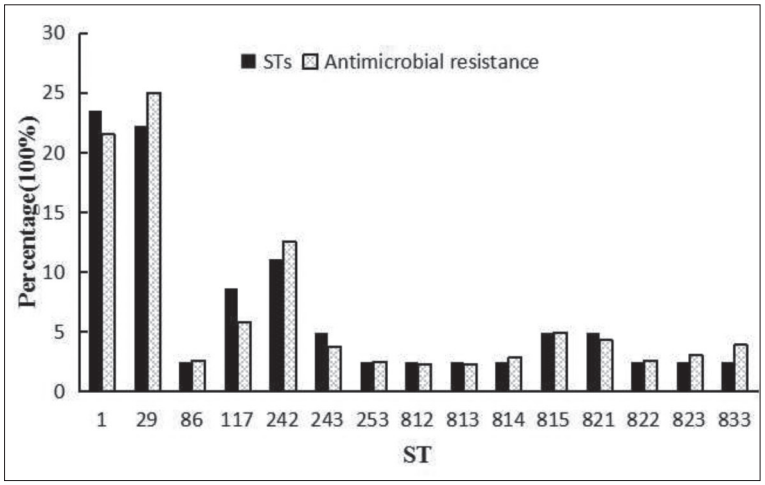


Fig 3. Distribution of *S. suis* STs and antimicrobial resistance among each of these STs. STs: frequency (%) = number of strains belonging to a certain ST/total number of strains ×100%; Antimicrobial resistance: incidence (%) = number of resistances to all tested antimicrobial agents among strains of a certain ST/total number of resistances to all tested antimicrobial agents across all strains×100%

DISCUSSION

In this study, the antimicrobial susceptibility of 81 isolates of *S. suis* from Southern China was tested against 42 antimicrobial agents. Testing of 42 antibiotics indicated that these isolates were differentially resistant to 22 (over 40%) and 11 (over 80%) antibiotics.

Previous reports suggested that *S. suis* strains had a high resistance to macrolides, lincosamides, tetracycline, and sulfonamides [11-15]. The *S. suis* isolates in this study showed a high resistance to macrolides, lincosamides, tetracycline, and sulfonamides, as well as a high resistance to quinolones and aminoglycosides.

Interestingly, a high resistance to foroxone (92.6%) and lomefloxacin (85.2%) was found in this study. The use of foroxone has been banned in veterinary medicine in China

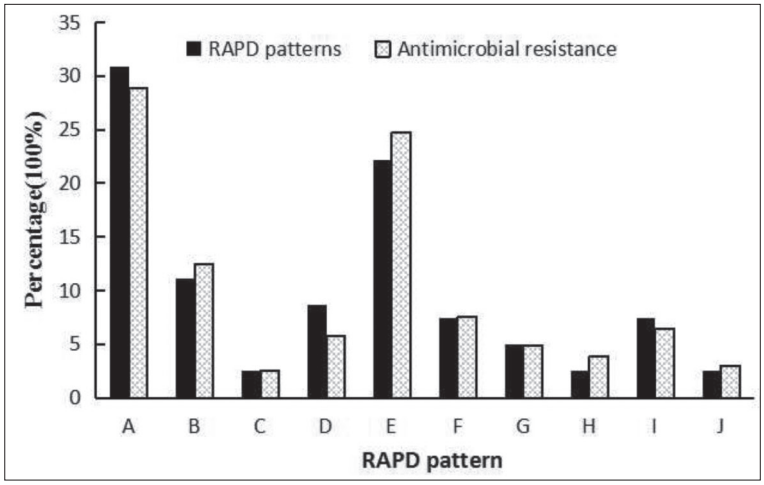
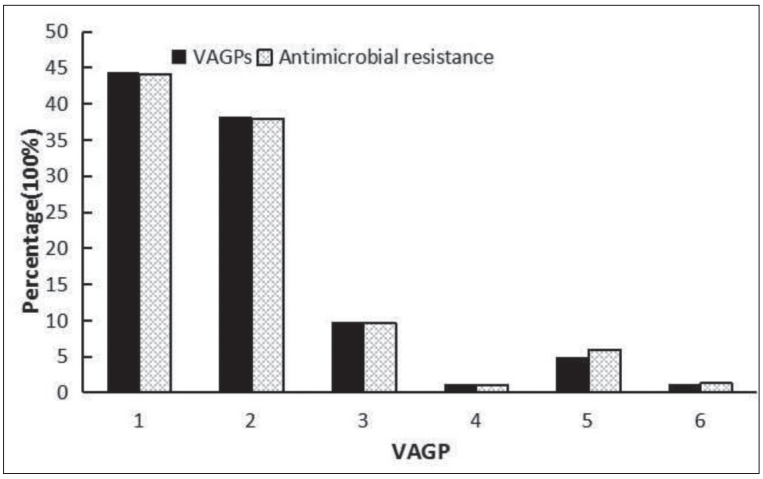


Fig 4. Distribution of *S. suis* RAPD patterns and antimicrobial resistance among each of these RAPD patterns. RAPD patterns: frequency (%) = number of strains belonging to a certain RAPD pattern/total number of strains ×100%; Antimicrobial resistance: incidence (%) = number of resistances to all tested antimicrobial agents among strains of a certain RAPD pattern/total number of resistances to all tested antimicrobial agents across all strains×100%

Fig 5. Distribution of *S. suis* VAGPs and antimicrobial resistance among each of these VAGPs. VAGPs: frequency (%) = number of strains belonging to a certain VAGP/total number of strains ×100%; Antimicrobial resistance: incidence (%) = number of resistances to all tested antimicrobial agents among strains of a certain VAGP/total number of resistances to all tested antimicrobial agents across all strains×100%



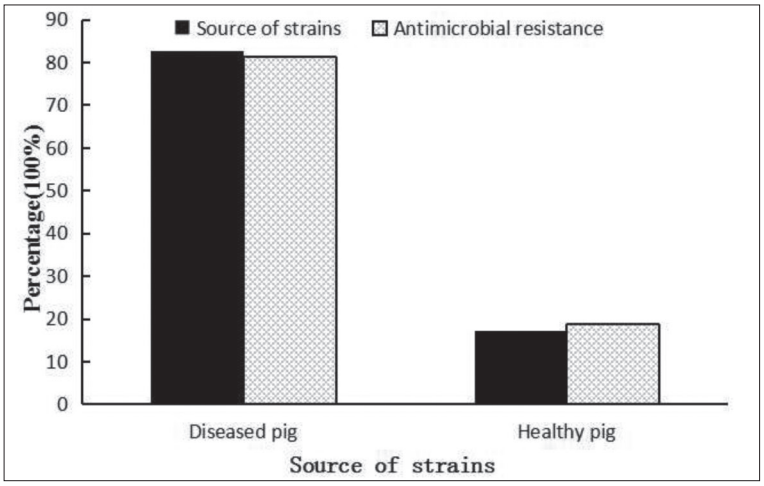


Fig 6. Distribution of source of strains and antimicrobial resistance in corresponding source of strains. Source of strains: frequency (%) = number of strains isolated from diseased pigs or healthy pigs/total number of strains ×100%; Antimicrobial resistance: incidence (%) = number of resistances to all tested antimicrobial agents among strains isolated from diseased pigs or healthy pigs /total number of resistances to all tested antimicrobial agents across all strains×100%

since 2002, while lomefloxacin was banned in 2015. Bischoff et al.[35] reported that antimicrobial resistance can be sustained when antimicrobial selective pressure is removed. The reason for this may be that resistance selection of bacteria can occur through a variety of mechanisms and is not always related to the use of specific antibiotics.

Previous reports suggested the resistance rate for β-lactams in *S. suis* was low [11,14,36]. In this study, the resistance rate of other β-lactams was relatively low except for cefixime, which indicates that β-lactams may be the primary drugs to treat the infection of *S. suis*.

Previous studies indicated that tetracycline-resistance was an important cofactor in the selection of resistance to macrolides/lincosamides [37,38]. In this study, 85.2% of the 81 isolates were resistant to tetracycline, 82.6% of which

were coresistant to macrolides and lincosamides. This indicated more frequent coresistance to tetracyclines and macrolides/lincosamides in *S. suis* isolates from Southern China.

The potential correlation between the antimicrobial resistance and serotypes, STS, RAPD patterns, VAGPs, or strain sources were examined,no statistically significant correlations between them were found (x^2-test, $P>0.05$).

In this study, 14 *S. suis* strains were isolated from healthy pigs, while 67 were isolated from diseased pigs. Seventy-three strains belonged to 6 distinct serotypes (2, 3, 7, 9, 19, and 28). The 8 remaining strains were NT. Six VAGPs, 10 RAPD patterns, and 15 STs were found among the 81 strains. The strains classified as same ST had the same serotype, VAGP, and RAPD patterns. All serotype 2 strains in the study belonged to VAGP1, but they were divided into 6 STs and 3 RAPD patterns. Serotype 2 strains with VAGP1/RAPD-A profiles were classified as ST1, ST86, ST812, and ST813. Serotype 9 strains with VAGP3/RAPD-F profiles were classified as ST243 and ST814. The 6 NT strains with an RAPD-I pattern were classified as ST821 and ST822; the 2 NT strains with an RAPD-J pattern were classified as ST823, but the 8 NT strains possessed 4 VAGPs. Accordingly, the MLST provided the highest resolution for *S. suis* strains among the 4 typing methods used in the study. There was a certain correlation among serotypes, STs, VAGPs, and RAPD patterns.

Serotype 2 strains were predominant in this study. These results are in line with previous reports [39,40]. All of these serotype 2 strains belonged to the ST1 clonal complex, indicating one route of clonal dissemination of these serotype 2 isolates. The ST1 clonal complex is the most widely distributed, possessing the highest pathogenicity and the largest number of isolates reported worldwide. In China, ST7 strains have been often isolated. ST7 is a SLV of ST1 (allelic profile 1, 1, 1, 1, 1, 1, 1) with increased virulence [41], but ST7 strains were not found in this study. However, 19 ST1 strains, 9 ST242 strains, and 2 ST253 strains were found. ST242 and ST253 are SLVs of ST7 (allelic profile 1, 1, 1, 1, 1, 1, 3). 4 serotype 2 strains classified as ST812 and ST813 were found; ST812 and ST813 are SLVs of ST1. The results show that *S. suis* strains in Southern China are continually evolving.

Serotype 2 strains possessed all 7 virulence genes, while serotypes 3, 7, and 9 possessed 5 virulence genes (lacking *epf* and *sly)*. The strains isolated from healthy pigs possessed 2-4 virulence genes (lacking *epf*, *sly,* etc.).

In this study, the 36 strains of *S. suis* serotype 2 were all classified as GDH Group I by the analysis of the *gdh* gene. As mentioned earlier, Group I consisted of highly virulent isolates. Therefore, these serotype 2 strains showed the molecular characteristics of highly pathogenic strains in VAGP, GDH sequence types, and MLST.

For the first time, this study provided an overall and systemic description of the correlation between the anti-microbial resistance and serotypes, STS, RAPD patterns, VAGPs, or strain sources in *S. suis* from Southern China,but, no significant correlation was observed. Significant differences in STs, RAPD patterns, and VAGPs were observed between strains isolated from healthy pigs and diseased pigs. β-lactams are still the most effective drugs to be used for the treatment of *S. suis* infections in Chinese veterinary clinics. eBURST analysis of *S. suis* isolates showed that the *S. suis* isolates in China are continually evolving. Therefore, increased surveillance of *S. suis* in piggeries must be considered. Detection of virulence-associated genes and determination of GDH and MLST can help predict the virulence of *S. suis* serotype 2 isolates.

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