Letter to the Editor

Genomic Characterization of a *Streptococcus suis* Serotype 2 Isolated from a Human Patient*

GUO Geng Lin[1,2,3,&], GAO Hua Sheng[4,&], WANG Zhuo Hao[1,2,3], TAN Zhong Ming[5], HAN Ming Xiao[4], CHEN Qi[4], DU Hong[4], ZHANG Wei[1,2,3,#], and ZHANG Hai Fang[4,#]

Streptococcus suis (*S. suis*) is a Gram-positive zoonotic pathogen. *S. suis* infection in humans commonly causes meningitis, septicemia, arthritis, and streptococcal toxic shock-like syndrome (STSLS)[1]. *S. suis* has 29 serotypes, of which *S. suis* serotype 2 (SS2) has the highest clinical isolation rate and strongest pathogenicity and causes most *S. suis* human infections. About 1,000 different sequence types (STs) were identified in *S. suis* based on multi-locus sequence typing (MLST). Among these, STs, ST1, and ST7 belong to serotype 2 and are the major hazards of *S. suis*[2]. In 1998 and 2005, two cases of SS2 human infection outbreak were reported in Jiangsu and Sichuan, China, causing 14 deaths and 38 deaths, respectively[3].

On July 29, 2019, a 75-year-old female patient was admitted to The Second Affiliated Hospital of Soochow University for treatment due to "fever accompanied by disturbance of consciousness for one day". The patient had high leukocyte and calcitonin, severe infection, septic shock, severe condition, and multiple organ damage. The patient was given anti-infection treatment. Unfortunately, the patient died after three days of rescue. Ethical approval was obtained from the ethics committee of Second Affiliated Hospital of Soochow University.

An *S. suis* isolate was obtained from the blood sample of this patient and identified by MALDI-TOF MS, 16S rRNA, and *gdh* (a genus-specific gene of *S. suis*). Subsequently, a *cps2I* (SS2 specific gene) gene was used to identify this isolate as serotype 2. Furthermore, we found that this isolate belongs to the ST7 type through scanning 7 housekeeping genes used in MLST. Whole genome sequencing is now a powerful tool to study the phylogenetics of bacterial pathogens. Total genomic DNA was extracted and sequenced using the Illumina NovaSeq PE150 platform (Illumina, https://www.illumina.com). This SS2 isolate was named SZ1908, and the whole genome sequence of SZ1908 was deposited in the Genbank database under accession number CP082948.1. SZ1908 contained a single, circular chromosome that is 2,137,458 base pairs (bp) long, with a genomic GC content of 41.29%. The chromosome carries 2029 protein-coding genes, 56 tRNA genes, and 12 rRNA genes (Supplementary Figure S1 available in www.besjournal.com).

To explore the genomic differences in this isolate with other *S. suis* isolates, all the 33 SS2 isolates obtained from Genbank were retrieved. A whole genome alignment was performed using the software Roary, and the core genes were extracted to build the phylogenetic tree using the Neighbor-joining method. Through association analysis with phenotypes such as virulence, sequence type, and isolation source, we found that SZ1908 belongs to the same clade as virulent ST7 SS2. However, it shows some obvious differences from the other human isolates (Figure 1).

To further analyze the genetic difference between SZ1908 and other SS2 isolates, we colinear aligned the genome of SZ1908 with those of three representative SS2 strains, including SC84 (an epidemic ST7 human isolate), P1/7 (an ST1 pig isolate), and T15 (classic avirulent strain). We found that the genomic backbone of SZ1908 is different

doi: 10.3967/bes2022.099

*This study was supported by the National Natural Science Foundation of China [82172332]; Gusu health youth talent of Suzhou [GSWS2019039, GSWS2020030]; the Science and Technology Program of Suzhou [SKY2021007]; Discipline Construction of The Second Affiliated Hospital of Soochow University [XKTJ-TD202001]; and Postgraduate Research & Practice Innovation Program of Jiangsu Province.

1. College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, Jiangsu, China; 2. Key Lab of Animal Bacteriology, Ministry of Agriculture, Nanjing 210095, Jiangsu, China; 3. OIE Reference Lab for Swine Streptococcosis, Nanjing 210095, Jiangsu, China; 4. Department of Clinical Laboratory, The Second Affiliated Hospital of Soochow University, Suzhou 215004, Jiangsu, China; 5. NHC Key Laboratory of Enteric Pathogenic Microbiology, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing 210009, Jiangsu, China

from that of T15, which has a large fragment transversion and rearrangement in the middle part. The genomic backbone of SZ1908 was much similar to that of the virulent SC84 and P1/7 with only one 100K insert and transversion. However, the genome of SZ1908 is much larger than that of P1/7 and SC84 (Figure 2). Also, the SS2 isolates belong to the same tidy clade as SZ1908 (Figure 1). The genomes of these three isolates are highly similar, with only one rearrangement in 600,000 to 700,000 bp. There are differences between single gene deletions and insertions, and through bioinformatic analysis, we found that this is the ICE, which is described below (Supplementary Figure S2 available in www.besjournal.com).

More than 100 virulence factors or virulence-associated genes have now been identified in *S. suis*. Among these genes is muramidase-released protein

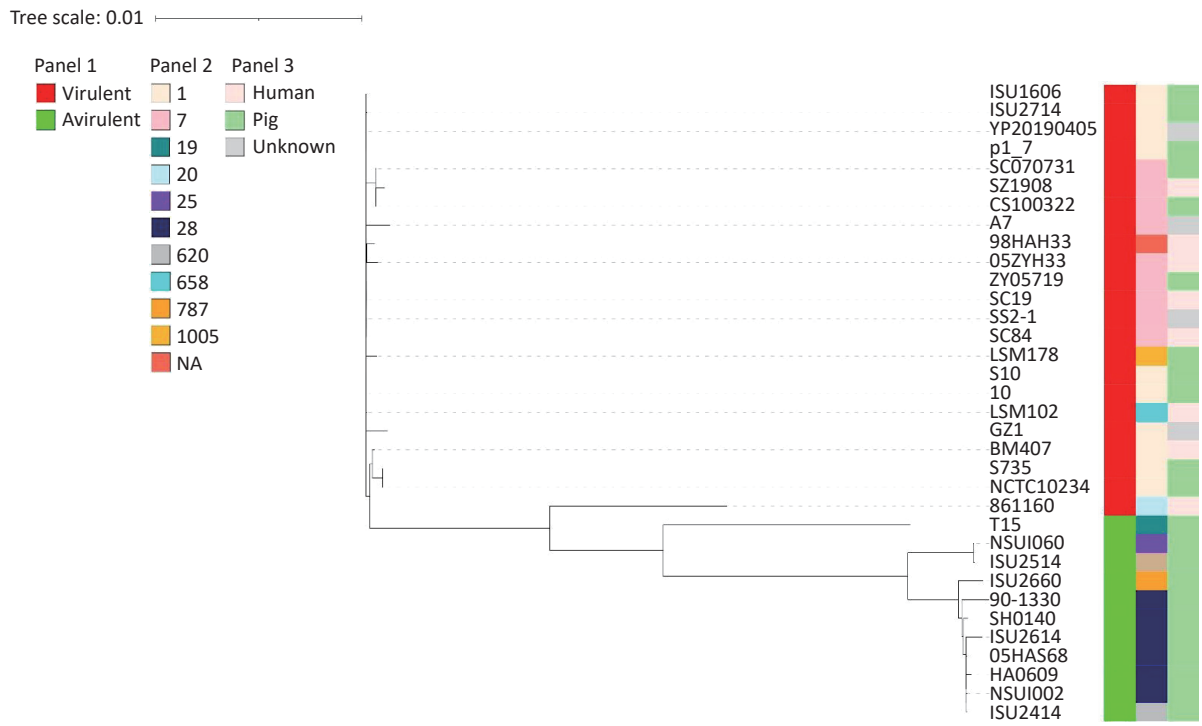


Figure 1. Phylogenic tree of SS2 whole genome sequence based on core gene alignment. The phenotype panel 1 is virulence, panel 2 is STs, and panel 3 is isolation source. The tree scales described the phylogenetic distance.

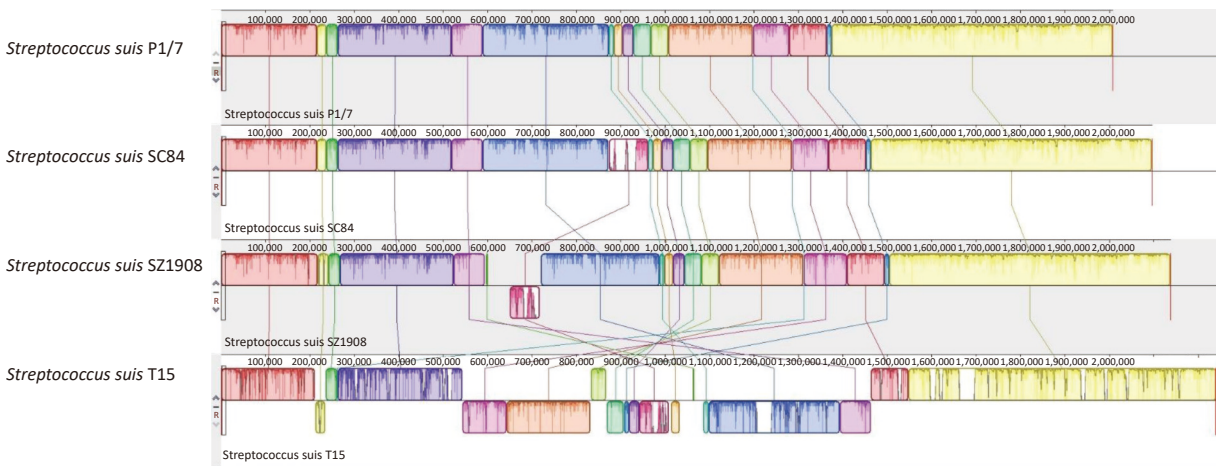


Figure 2. Colinear Alignment of the genome of SZ1908 with other classic SS2 strains.

(MRP), a cell wall anchored protein with a molecular weight of 136 kD and named MRP because it can be released from the cell wall after treatment of SS2 virulent strain with muramidase. MRP has been traditionally regarded as a virulence marker to identify the virulence of *S. suis*[4]. Li et al.[5] found that both virulent and avirulent SS2 isolates contain the gene *mrp* although there are some differences. The gene *mrp* could be divided into two parts, a conserved region and a variable region. The variable region is the main factor involved in the virulence of SS2 and the phylogenetic tree constructed based on this region could separate SS2 strins into two clades by virulence[5]. In this study, we extracted the *mrp* gene of SZ1908 and those of the other SS2 isolates deposited in Genbank and constructed a phylogenic tree of *mrp* genes. We found that the *mrp* gene sequence of SZ1908 was much closer to those of the classical SS2 avirulent isolates and not in the same clade with those of other human isolates of the SS2 strains (Figure 3). These findings indicate that some other genes could be better markers of *S. suis* virulence. The extracellular factor, suilysin (SLY), also found in the isolate, can be used as a virulence

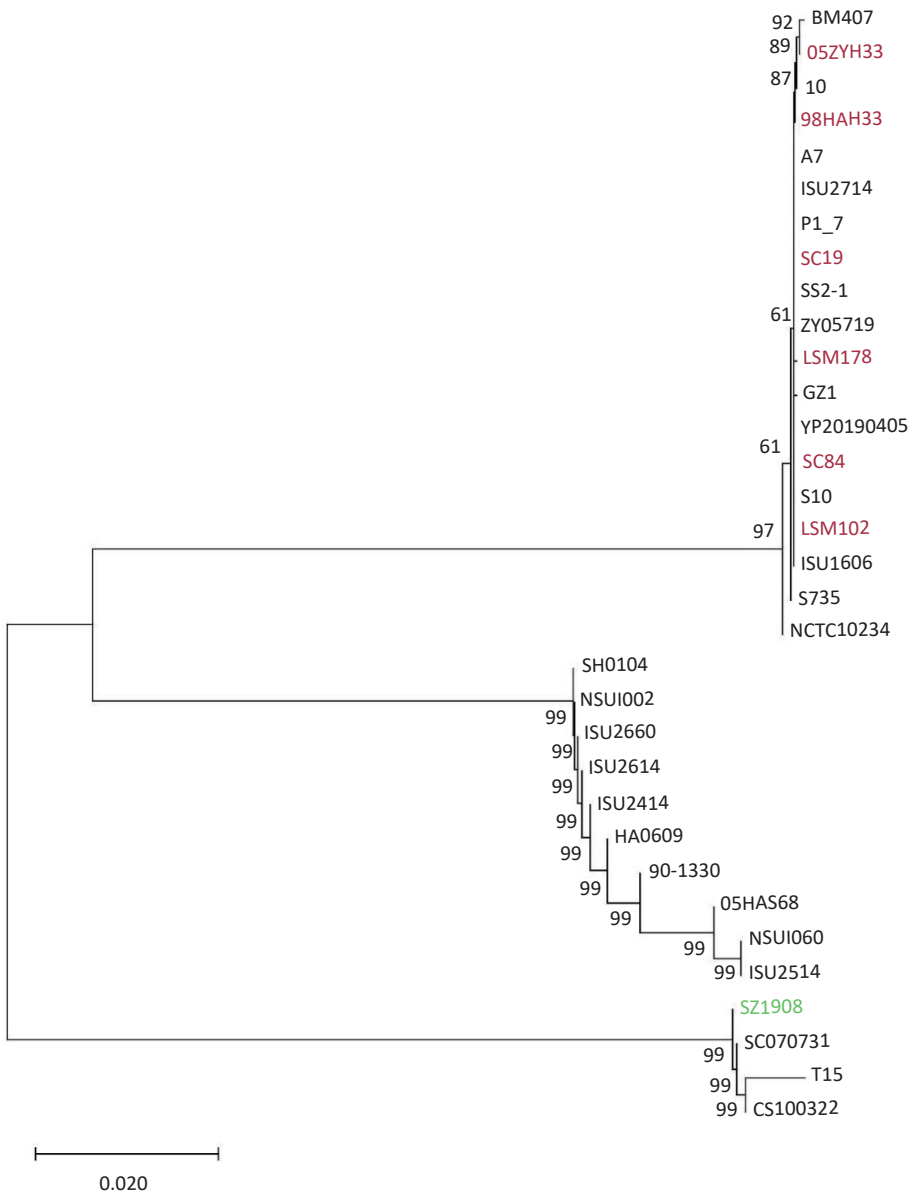


Figure 3. Phylogenic tree of *mrp* sequence of SS2 strains. All human isolate SS2 strains were marked by red, and this isolate was marked by green.

marker of *S. suis*. Other potential virulence markers of *S. suis* also found in the isolate include the type I RM system S protein (SSU1589), predicted copper ATPase (SSU0207) proposed by Gottschalk[6], and the major pili subunit SBP2.

Antimicrobial resistance is one of the most important problems in controlling bacterial diseases. In *S. suis*, antimicrobial resistance is not that severe. Resistance to tetracyclines, macrolides, and aminoglycosides, especially tetracycline, and erythromycin, is the most prevalent and is associated with resistance gene *tet(O)* and *erm(B)*[7]. Through detecting the minimum inhibitory concentration (MIC) of multiple antimicrobials to SZ1908, we found that SZ1908 is resistant to several antibiotics, including tetracycline and erythromycin (Supplementary Table S1 available in www.besjournal.com). After WGS, the antimicrobial resistance genes (ARGs) were scanned by the online server Resfinder, leading to the identification of seven ARGs, namely *msr(D)*, *erm(B)*, *ant(6)-Ia*, *aph(2')-III*, *tet(O)*, *tet(40),* and *mef(A),* and the loci of these seven ARGs in the SZ1908 genome are shown in Supplementary Table S2 available in www.besjournal.com. The integrative conjugative element (ICE) is an important mobile genetic element in bacteria that could facilitate the migration of antibiotic resistance determinants[8]. Several ICEs have been reported in *S. suis* since 2012, most of which carried tetracycline and erythromycin resistance genes, which is consistent with the prevalence of antibiotic resistance in *S. suis*. In SZ1908, a whole length of 189637 bp ICE was identified in the genome, stretching from 561165 to 750802, which is the extra part in SZ1908 without a corresponding part in P1/7 and SC84. Notably, all the seven ARGs were located in this ICE (Supplementary Figure S3 available in www.besjournal.com). Considering that SZ1908 was isolated from a patient, ICE could be an emerging risk to public health.

This study reports a human infection case of *S. suis*, which is an important zoonotic pathogen. After genomic analysis, we found that the genome of this isolate is different from those of previous human isolates, and the classic *S. suis* virulence marker *mrp* gene could not fit this isolate. Furthermore, this isolate exhibited multidrug resistance and had seven different ARGs located in an integrative conjugative element, which could meditate the migration of antimicrobial resistance. This study has generated information useful for understanding the epidemiology of *S. suis* and its implications for public health.

Data Availability The whole genome sequence of SZ1908 was deposited in the GeneBank database under accession number CP082948.1.

Conflict of Interests The authors declare no conflict of interest.

[&]These authors contributed equally to this work.

[#]Correspondence should be addressed to ZHANG Hai Fang, E-mail: haifangzhang@suda.edu.cn, haifangzhang@sina.com; ZHANG Wei, E-mail: vszw@njau.edu.cn

Biographical notes of the first authors: GUO Geng Lin, male, born in 1995, PhD Student, majoring in bacteria pathogen pathogenesis and genomics; GAO Hua Sheng, male, born in 1977, majoring in clinical microbiologist.

Received: January 17, 2022;

Accepted: April 11, 2022

REFERENCES

1. Feng YJ, Zhang HM, Wu ZW, et al. *Streptococcus suis* infection: an emerging/reemerging challenge of bacterial infectious diseases? Virulence, 2014; 5, 477-97.
2. Haas B, Grenier D. Understanding the virulence of *Streptococcus suis*: a veterinary, medical, and economic challenge. Méd Mal Infect, 2018; 48, 159–66.
3. Yu HJ, Jing HQ, Chen ZH, et al. Human *Streptococcus suis* outbreak, Sichuan, China. Emerg Infect Dis, 2006; 12, 914–20.
4. Fittipaldi N, Segura M, Grenier D, et al. Virulence factors involved in the pathogenesis of the infection caused by the swine pathogen and zoonotic agent *Streptococcus suis*. Future Microbiol, 2012; 7, 259–79.
5. Li Q, Fu Y, Ma CF, et al. The non-conserved region of MRP is involved in the virulence of *Streptococcus suis* serotype 2. Virulence, 2017; 8, 1274–89.
6. Scherrer S, Rosato G, Serrano NS, et al. Population structure, genetic diversity and pathotypes of *Streptococcus suis* isolated during the last 13 years from diseased pigs in Switzerland. Vet Res, 2020; 51, 85.
7. Palmieri C, Magi G, Mingoia M, et al. Characterization of a *Streptococcus suis tet*(O/W/32/O)-carrying element transferable to major streptococcal pathogens. Antimicrob Agents Chemother, 2012; 56, 4697–702.
8. Liu M, Li XB, Xie YZ, et al. ICEberg 2. 0:an updated database of bacterial integrative and conjugative elements. Nucleic Acids Res, 2019; 47, D660–5.

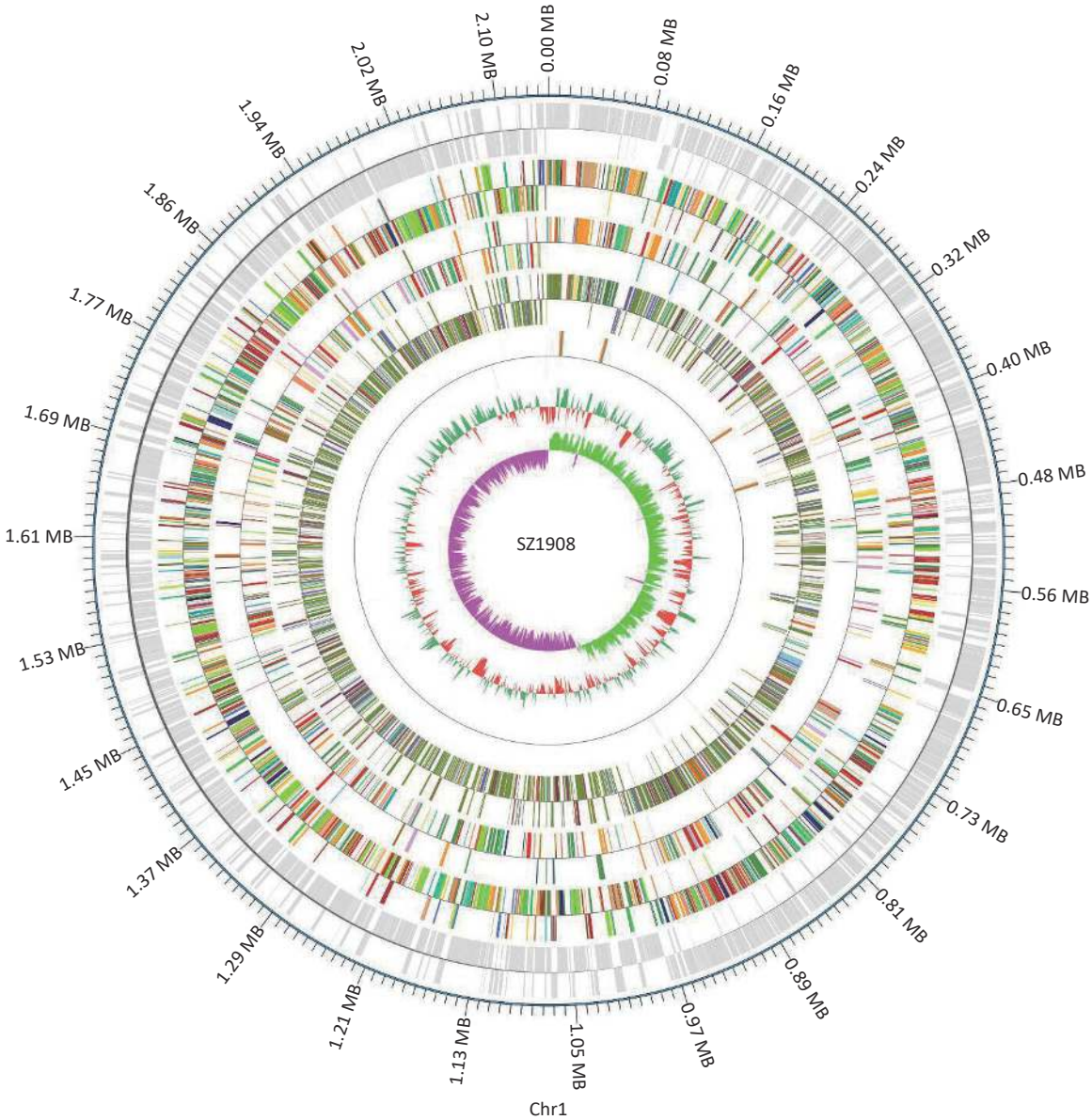
Supplementary Table S1. Sensitivity of SZ1908 to antibiotics

Antibiotics	Results of MIC	Sensitivity	Breakpoint (CLSI 2021)		
			S	I	R
Amoxicillin	≤ 0.25	Sensitive	0.25	0.5--4	8
Cefepime	≤ 0.5	Sensitive	1	2	4
Cefotaxime	≤ 0.5	Sensitive	1	2	4
Chloramphenicol	4	Sensitive	4	8	16
Clindamycin	> 1	Resistance	0.25	0.5	1
Erythromycin	> 4	Resistance	0.25	0.5	1
Meropenem	≤ 0.0625	Sensitive	0.5	--	--
Tetracycline	> 8	Resistance	2	4	8
Vancomycin	≤ 0.5	Sensitive	1	--	--
Levofloxacin	≤ 0.5	Sensitive	2	4	8
Linezolid	≤ 1	Sensitive	2	--	--
Penicillin	0.0625	Sensitive	0.12	0.25--2	4

Note. S: susceptible; I: intermediate; R: resistant.

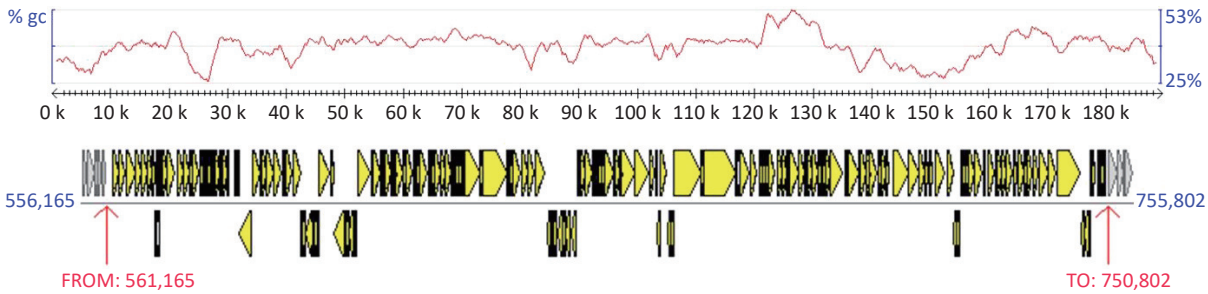
Supplementary Table S2. Loci of antimicrobial resistance genes in the SZ1908 genome

Resistance gene	Position in SZ1908	Phenotype
aph(3')-III	646676..647470	Aminoglycoside resistance
ant(6)-Ia	648102..649010	Aminoglycoside resistance
mef(A)	595139..596356	Macrolide resistance
msr(D)	596476..597939	Macrolide, Lincosamide and Streptogramin B resistance
erm(B)	649315..650052	Macrolide resistance
tet(40)	683094..684314	Tetracycline resistance
tet(O)	681124..683043	Tetracycline resistance



Supplementary Figure S1. Circular representation of the genome of SZ1908. From the outer to inner layers, the circle shows genes, COG, KEGG, GO, and ncRNA.

Supplementary Figure S2. Colinearize Alignment of the genome of SZ1908 with SC070731 and CS100322.



Supplementary Figure S3. Character of ICE containing 7 ARGs in SZ1908.