scientific reports

OPEN

Genomic analysis of a clinical *Streptococcus suis* ST1 isolate from CSF reveals antimicrobial resistance, virulence, and an evolutionary link to ST7

Jie Jiang[1], Wenlong Duan[2] & Liang Liang[1]✉

***Streptococcus suis* (*S. suis*) has emerged as an important zoonotic pathogen that can cause serious human infections. *S. suis* sequence type 1 (ST1) is predominant in sporadic human infections. However, few studies have characterized *S. suis* ST1 isolated directly from human clinical specimens.*Streptococcus suis* 366 was isolated from the cerebrospinal fluid (CSF) of a patient with purulent meningitis. Antimicrobial susceptibility testing and whole-genome sequencing were performed. Detailed functional annotations (CARD, VFDB, GO, KEGG, and COG), along with pangenome, phylogenetic, and comparative genomic analyses, were conducted using rigorously curated bioinformatics tools. The clinical strain belonged to ST1 and was resistant to erythromycin, clindamycin, and tetracycline. 5 mobile genetic elements were detected but no plasmids were detected. The resistance phenotype was consistent with those associated with 2 resistance genes (tet-(o) and ErmB). The isolate was predicted to be highly pathogenic and harboured 4 virulence genes (hasC, cpsF, neuB, and pavA). However, no resistance genes or virulence genes were identified on 5 mobile genetic elements. In the biological process category, terms related to 'cellular process' and 'metabolic process' were the most prominently enriched, suggesting that the gene set is primarily involved in cellular adaptation. The most significantly enriched pathway was the 'ko02010 ABC transporter pathway'. The functions of the genes in the strain are concentrated mainly in categories such as E (amino acid transport and metabolism), G (carbohydrate transport and metabolism), J (translation, ribosomal structure, and biogenesis), and R (general function prediction only). Pangenome analysis revealed that ST1 and ST7 shared a large number of core genes. Phylogenetic and comparative analysis revealed that ST1 and ST7 were closely related and that there was an evolutionary connection. This study provides significant insights into the genomic characteristics associated with *S. suis* ST1, enhancing the understanding of this zoonotic pathogen from human CSF. Furthermore, the findings enhance the understanding of the evolutionary connection between the ST1 and ST7 strains.**

Keywords Streptococcus Suis, Purulent meningitis, ST1, Cerebrospinal fluid, Molecular characterization

Streptococcus suis (*S. suis*) is a gram-positive bacterial pathogen in the *Streptococcus* genus that causes substantial economic losses in the global pig industry[1]. The natural habitat of *S. suis* is the upper respiratory tract of pigs. It can also be found in the digestive tract and genitals[2]. *S. suis* causes outbreaks of meningitis, septicaemia, and pneumonia in neonatal piglets and adult pigs[3,4]. Moreover, *S. suis* is recognized as an emerging zoonotic pathogen that can cause meningitis, endocarditis, arthritis, sepsis, hearing loss, skin lesions and even death in humans[5–7]. *S. suis* human infections have raised great public concern worldwide, and the majority of human infections occur in Thailand, Vietnam, and China[7]. *S. suis* sequence type (ST) 1 strains are the most frequently isolated from human cases worldwide, and ST1 has been predominant in sporadic human infections[8,9]. Multilocus

[1]Department of Clinical Laboratory, The People's Hospital of Guangxi Zhuang Autonomous Region (Guangxi Academy of Medical Science), Nanning 530021, Guangxi Autonomous Region, China. [2]Department of Emergency, The People's Hospital of Guangxi Zhuang Autonomous Region (Guangxi Academy of Medical Science), Nanning 530021, Guangxi Autonomous Region, China. ✉email: liangliang5653@163.com

Antimicrobial groups	Antimicrobial agent	MIC	Interpretation
Aminoglycosides	Penicillin	0.06	Sensitive
	Ampicillin	≤0.25	Sensitive
Cephems	Ceftriaxone	≤0.5	Sensitive
	Cefotaxime	≤0.5	Sensitive
	Cefepime	≤0.5	Sensitive
Glycopeptides	Vancomycin	≤0.5	Sensitive
Fluoroquinolones	Levofloxacin	≤0.5	Sensitive
Oxazolidinones	Linezolid	≤1	Sensitive
Carbapenems	Ertapenem	≤1	Sensitive
	Meropenem	≤0.06	Sensitive
Phenicols	Chloramphenicol	4	Sensitive
Macrolides	Erythromycin	>4	Resistant
Lincosamides	Clindamycin	>1	Resistant
Tetracyclines	Tetracycline	>8	Resistant

Table 1. Antibiogram profiling of *Streptococcus suis* 366.

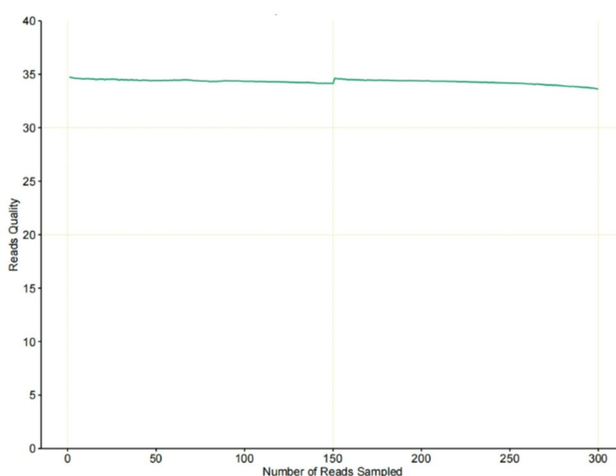

Fig. 1. Quality scores across all bases.

sequence typing (MLST) analysis revealed that the ST 7 strains were derived from ST1 by the acquisition of 5 genomic islands[10].

Most *S. suis* studies have been conducted on isolates from porcine sources[11]. However, studies on strains directly isolated from human CSF is rare. The genomic characteristics of *S. suis* strains directly isolated from human CSF remain poorly understood. Here, we sequenced the genome of an *S. suis* ST1 strain isolated from the CSF of a patient with severe meningitis. The findings of this study provided significant insight into the genomic characteristics, prediction of the pathogenic capacity, antimicrobial gene repertoire, putative virulence genes, and phylogenetic evolution of the isolate, enhancing the understanding of this zoonotic pathogen from human CSF.

Results

Antimicrobial susceptibility profiles

We identified resistance to erythromycin, clindamycin and tetracycline in the strain, along with susceptibility to penicillin, ampicillin, ceftriaxone, cefotaxime, cefepime, vancomycin, levofloxacin, linezolid, ertapenem, meropenem, and chloramphenicol (Table 1).

Genome composition analysis

The base quality scores for all positions were primarily concentrated in the green background area shown in Fig. 1, demonstrating high and consistent sequencing quality. A total of 15,350,328 reads were obtained after read cleaning. From these clean reads, the entire bacterial genome was successfully assembled into 34 contigs with a total length of 2,028,199 bp, a GC content of 41.21% and an N50 value of 131,426 bp (Table 2). The BUSCO (Benchmarking Universal Single-Copy Orthologues) assessment demonstrated high completeness in the genome assembly, with 96.6% of BUSCOs identified as complete, 2.6% as fragmented, and 0.9% as

Assembly statistics	
GenBank accession number	JBLMKP000000000
Assembly program	SPAdes genome assembler (v3.5.0)
Total Reads Count	15,350,328
Contig number	34
Max Length	305,932
Average Length	59652.91
All Length	2,028,199
N50	131,426
BUSCO	
Complete BUSCOs	96.60%
Fragmented BUSCOs	2.60%
Missing BUSCOs	0.90%
GC Ratio	0.41
Assembly level	contig
Annotation information	
Annotation program	NCBI Prokaryote Genome Annotation Pipeline
Genes	1982
Protein-coding	1933
RNA	49

Table 2. Assembly and annotation summary statistics.

missing[12]. The genome assembly had an assembly depth of 200×, indicating that the genome was well assembled (Supplementary Table S1). The genome contains a total of 1,982 genomic features, including 1,933 coding genes and 49 RNA genes (3 5 S rRNA genes, 3 16 S rRNA genes, 1 23 S rRNA gene, 41 tRNA genes, and 1 ncRNA gene) (Supplementary Table S2). No plasmid was detected in the strain. MobileElementFinder[13] detected 5 mobile genetic elements scattered across the genome, divided into 4 Insertion sequence(at contig 5, 6,14,21, respectively) and 1 Composite transposon(at contig6) (Supplementary Table S3). Analyses of the CRISPR–Cas system revealed one CRISPR array at contig 4 (CRISPR position 168638–168782, DR length = 43, number of spacers = 1), 1 CRISPR array at contig 16 (CRISPR: position 13657–13780, DR length = 32, number of spacers = 1) and two Cas clusters (CAS3_TypeI). Based on clustering of allele genes (aroA, cpn60, dpr, gki, mutS, recA and thrA) with those of *Streptococcus suis* strains, in silico analysis of the assembled genome indicated that this strain was MLST sequence type 1 (ST1). The isolate was predicted to be pathogenic (probability of being a human pathogen = 0.921 out of 1.0, indicating greater pathogenicity). A circular genome map showing other genomic features is presented in Fig. 2.

CARD annotation of *Streptococcus suis* 366

Two antimicrobial resistance genes, ermB and tet-(O), were detected in the genome using the Resistance Gene Identifier with the Comprehensive Antibiotic Resistance Database (CARD)[14].Gene ermB confers resistance to erythromycin and clindamycin via antibiotic target alteration. Erm proteins dimethylate a single adenine in nascent 23 S rRNA, which is part of the large (50 S) ribosomal subunit[15]. As a consequence of methylation, binding of erythromycin to its target is impaired. The overlapping binding sites of macrolides and lincosamides in 23 S rRNA account for cross-resistance to the 2 classes of drugs[16]. Gene tet-(O) confers tetracycline resistance via antibiotic target protection. The tet(O) is a paralogue of the translational GTPase EF-G 6 and actively removes tetracycline from the ribosome in a GTP-hydrolysis-dependent fashion[17]. Both AMR genes were located at contig 9. No AMR genes were located on a mobile genetic element.

VFDB annotation results for *Streptococcus suis* 366

The genome of *Streptococcus suis* 366 harboured 4 virulence-associated genes, including *hasC*, *cpsF*, *neuB*, and *pavA* (Table 33). *hasC*, *cpsF* and *neuB* were related to capsule synthesis. There are more than 20 proposed virulence factors contributing to the pathogenesis of *S.suis* infections, including the capsular polysaccharide (CPS)[18]. However, the role in the pathogenesis of *S. suis* infections of most putative virulence factors (other than CPS) described so far remains to be confirmed[19]. The critical virulence factor described is the CPS[20].CPS facilitates the survival of the strain in the bloodstream and the sialic acid component located in the terminal position of CPS is likely to be responsible for the antiphagocytosis[18,21]. These 4 virulence genes were located at separate contigs: hasC at contig 1, cpsF and neuB at contig 15, and pavA at contig 10. No virulence genes were located on mobile genetic elements.

Gene Ontology (GO) annotation of *Streptococcus suis* 366

To elucidate the biological functions of the differentially expressed genes, GO enrichment analysis was performed[22]. A bar plot was constructed to visualize the enriched terms across the biological process, cellular component, and molecular function categories (Fig. 3). In the biological process category, terms related to 'cellular process'

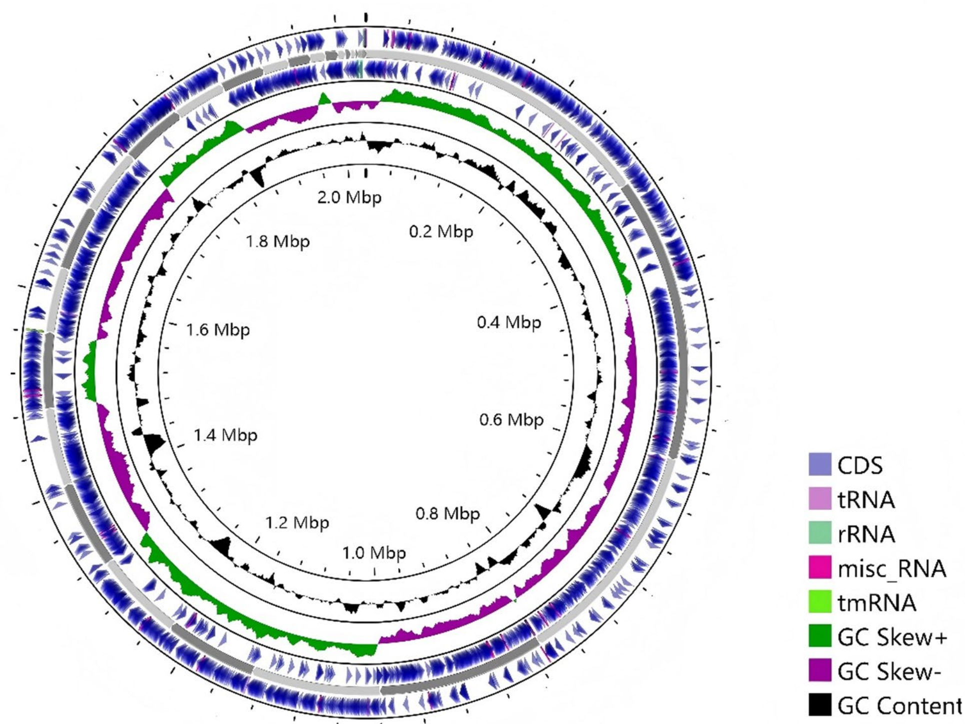

Fig. 2. Circular representation of the genome of highly pathogenic *Streptococcus suis* strain 366. The innermost circle represents the genomic sequence coordinates. Moving outwards, the circles represent GC content, GC skew (GC skewness, measuring the relative abundance of G and C, used to mark the starting and ending points on circular chromosomes), reverse-strand genes and forward-strand genes.

Gene ID	VFG Symbol	VF Information	VF Name	Function
ctg00001_00160	hasC	UDP-glucose pyrophosphorylase	Hyaluronic acid Capsule	Antiphagocytosis; Adherence; Tissue invasion
ctg00015_01831	cpsF	polysaccharide biosynthesis protein CpsF	Capsule	Antiphagocytosis; Serum resistance
ctg00015_01843	neuB	N-acetyl neuramic acid synthetase NeuB	Capsule	Antiphagocytosis; Serum resistance
ctg00010_01446	pavA	Fibronectin-binding protein-like protein A	Fibronectin-binding protein	Adherence; Non-fimbrial adhesin; Cell wall anchored protein

Table 3. Virulence genes in isolate *Streptococcus suis* 366.

(Count = 282) and 'metabolic process' (Count = 261) were the most prominently enriched, suggesting that the gene set is primarily involved in cellular adaptation. For molecular function, genes were significantly enriched in 'catalytic activity' (Count = 204), suggesting that the genes predominantly encoded enzymes. With respect to cellular components, 'protein-containing complex' (Count = 67) was highly represented, indicating that the phenotype associated with the gene set is largely mediated by stable, multi-subunit macromolecular machines in which at least one component is a protein. The complete GO annotations are provided in Supplementary Table S4.

KEGG annotation of *Streptococcus suis* 366

The results of the alignment of the gene sets with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database[23] are shown in Fig. 4. KEGG pathway analysis revealed that the differentially expressed genes (DEGs) were significantly enriched in terms related to five major categories: cellular processes, environmental information processing, genetic information processing, metabolism and organismal systems. Several pathways were significantly enriched. The most prominent among these were 'membrane transport' (Count = 130), 'carbohydrate metabolism' (Count = 152), and 'overview' (Count = 115). In the membrane transport category, the most significantly enriched pathway was the 'ko02010 ABC transporters pathway', with 86 DEGs mapped to this pathway. The ABC (ATP binding cassette) transporter is among the active transport systems of cells and is known as the binding-lipoprotein-dependent transport system in gram-positive bacteria[24]. This pathway

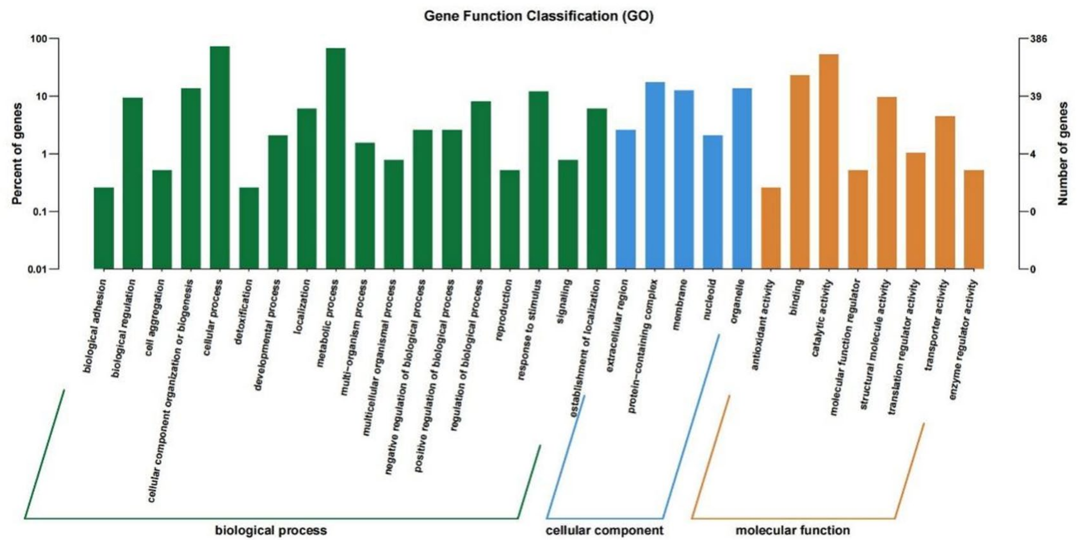

Fig. 3. Gene function classification of the *Streptococcus suis* 366 genome.

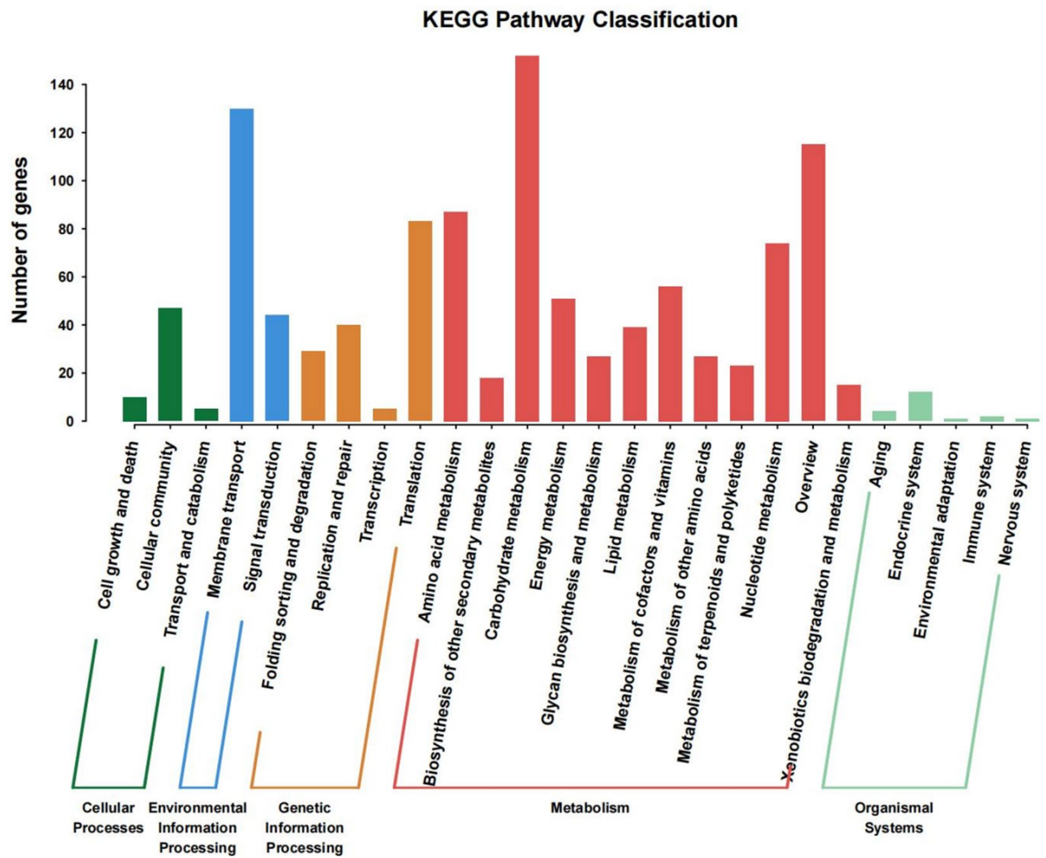

Fig. 4. KEGG pathway classification of the *Streptococcus suis* 366 genome.

is known to play a pivotal role in environmental information processing and membrane transport. ABC transporters function as either importers, bringing nutrients and other molecules into cells, or exporters that pump toxins, drugs and lipids across membranes[25,26]. The complete KEGG pathway annotation is provided in Supplementary Table S5.

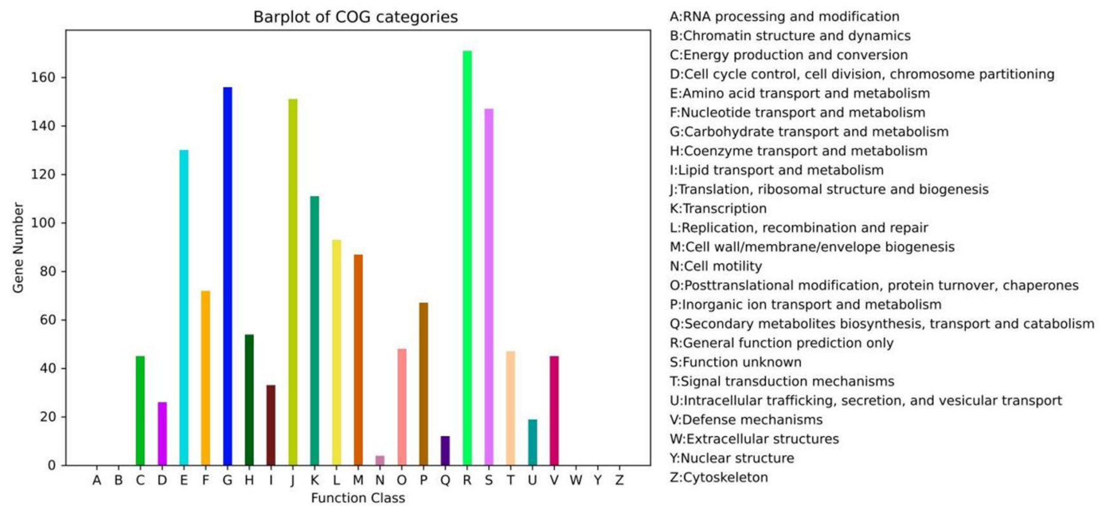

Fig. 5. COG annotation results for the *Streptococcus suis* 366 genome. Different colours represent distinct COG annotation categories. All COG annotation categories are labelled with letters, as shown on the right side of the figure.

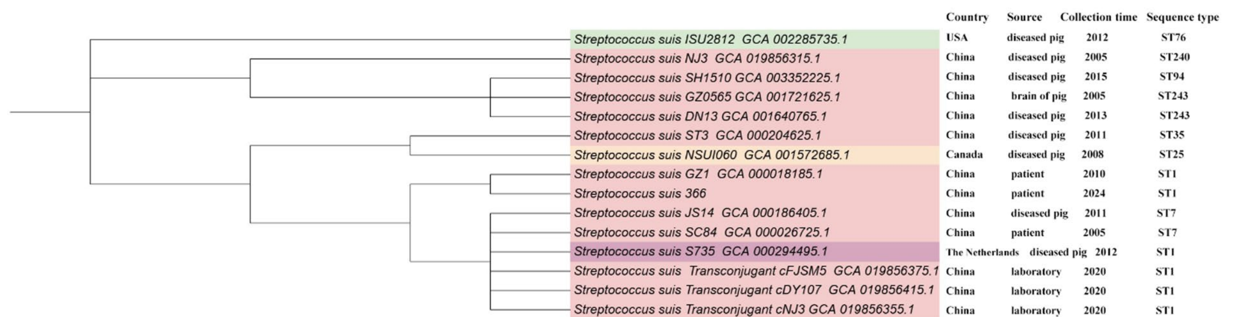

Fig. 6. Phylogenetic relationship between *Streptococcus suis* 366 and the other *Streptococcus suis* strains based on 16 S rRNA. Different colours represent the different geographical origins of those strains.

Gene function annotation

Functional annotation was performed by alignment with the Cluster of Orthologous Groups of proteins (COG) database[27] to analyse and infer gene functions. The COG annotation results for the isolate are shown in Fig. 5. The results indicated that the genome participated in various aspects of bacterial life processes. The functions of the genes in the *Streptococcus suis* 366 strain obtained in this study were mainly concentrated in categories such as E (amino acid transport and metabolism), G (carbohydrate transport and metabolism), J (translation, ribosomal structure, and biogenesis), and R (general function prediction), which are biologically significant for maintaining bacterial metabolism. Moreover, the functions of 147 genes were unknown and were concentrated in category S. The complete COG annotation is provided in Supplementary Table S6.

Phylogenetic analysis based on 16 S rRNA

Phylogenetic analysis based on 16 S rRNA revealed that *Streptococcus suis* GZ1 (GCA 000018185.1) was the closest *Streptococcus suis* 366 relative[18](Fig. 6). These strains were isolated from various countries, and the majority originated from China. Detailed information on these bacterial strains is provided in Supplementary Table S7.

Analysis of the pangenome

The results of the pangenome analysis of the *S.suis* 366 strain and its 7 closest relatives are presented in Fig. 7. Detailed information on related bacterial strains is provided in Supplementary Table S8. The pangenome of these

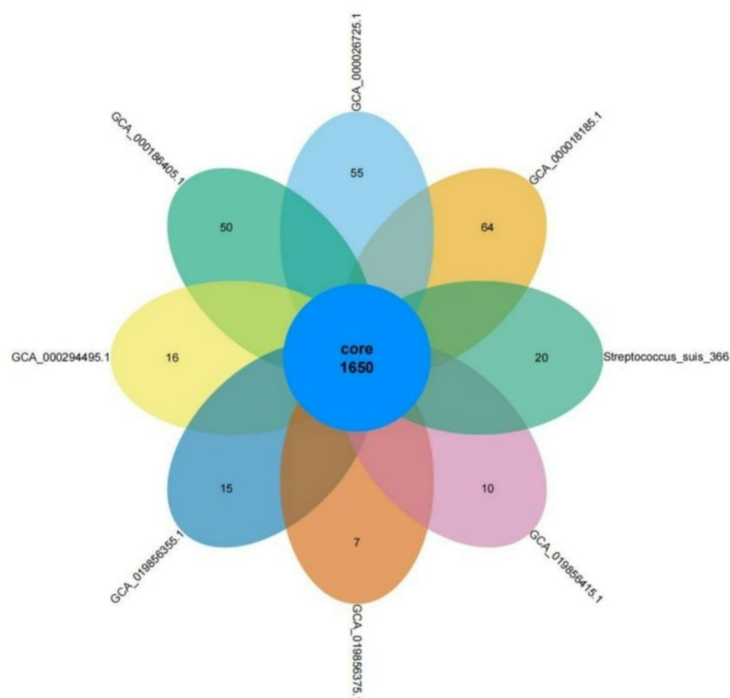

Fig. 7. Flower plot of homologous genes. The figure shows the numbers of common and unique orthologue clusters. The flower heart indicates the orthologue cluster in which all the strains are present, and the petals indicate those unique to each strain.

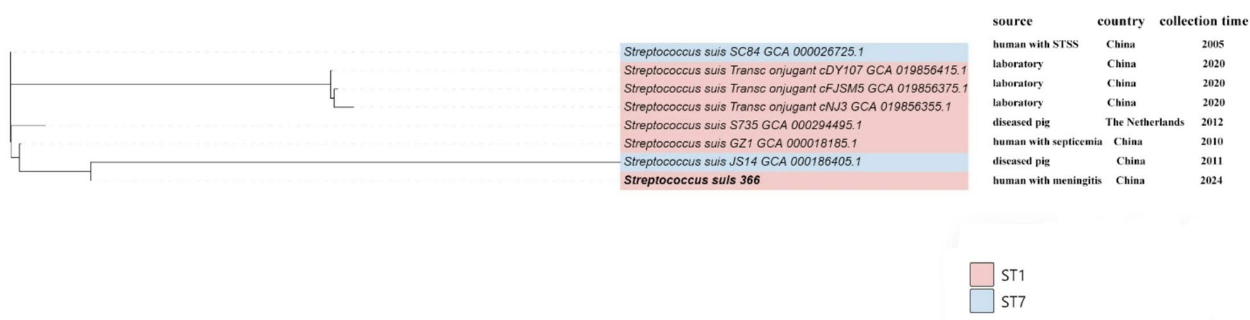

Fig. 8. Phylogenetic tree constructed based on the core-genome SNPs using the neighbour–joining method with the FastTree tool and visualized using the interactive Tree of Life tool. STSS: streptococcal toxic shock-like syndrome.

strains comprised a total of 2300 genes. Among them, 1650 core genes were shared by the 8 strains. In addition, there are 413 shell genes and 237 cloud genes.

Phylogenetic analysis of *Streptococcus suis* 366 based on SNPs

The resolution power of the 16 S rRNA analysis is limited due to the predominance of conserved sites in sequences, not giving enough evolutionary information for the analysis and restricting the identification at the genus level[28]. Therefore, phylogenetic analysis based on SNPs was performed. Phylogenetic analysis based on SNPs revealed *Streptococcus suis* JS14 (GCA 0000186405.1) as the closest *Streptococcus suis* 366 relative (Fig. 8). Among the 8 strains, the ST1 type was dominant. ST1 and ST7 were closely related in terms of evolution. Some of the strains were derived from diseased pigs, and some were from humans. Compared to the 16 S rRNA single-gene phylogenetic tree, the genome-wide SNP-based phylogeny provides a more resolved and accurate representation of evolutionary relationships.

Comparative genomic analysis with the closest pathogenic strains

Multi-genome comparisons among *S. suis* strains GZ1, 366, and JS14 are shown in Fig. 9. The highly pathogenic strain ST1 GZ1 was isolated from a patient with septicemia in China. The genome of highly pathogenic strain

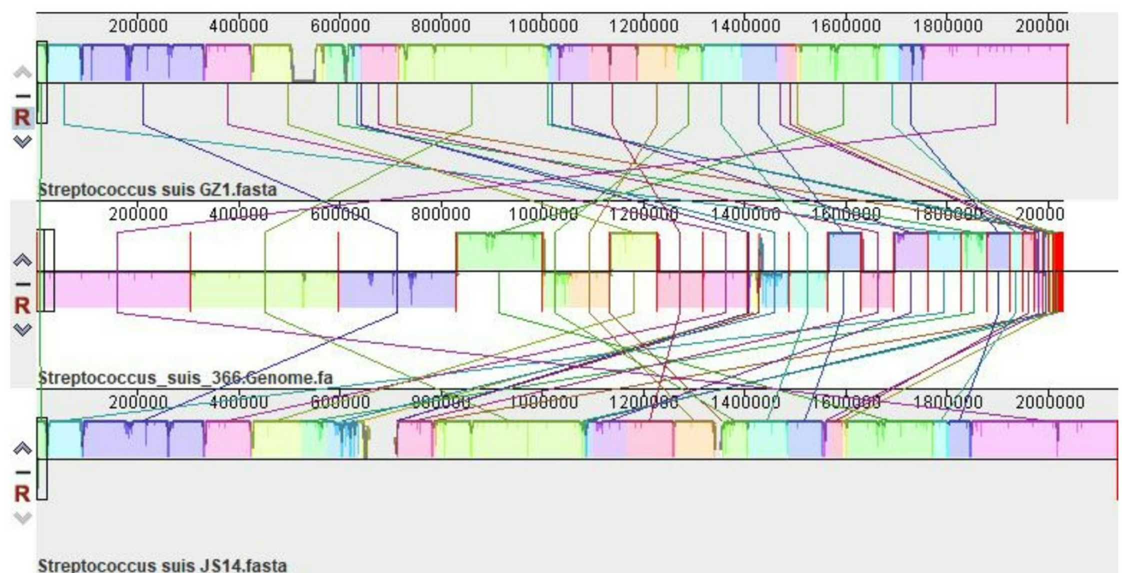

Fig. 9. Multi-genome comparisons among *S. suis* strains GZ1, 366, and JS14, performed using the Mauve software. Each color region refers to a locally collinear block (LCB). Colors are arbitrarily assigned to each LCB by the software. Vertical peaks in each LCB denote the variance of conservation. LCBs below the centerline of genomes are in reverse complement orientation.

ST1 GZ1 is a single circular chromosome of 2,038,034 bp with a GC content of 41.44% (CP000837). No plasmids were found and the chromosome has 1987 predicted coding sequences (CDSs)[9]. ST7 JS14 was isolated from articulation of a diseased pig in Jiangsu Province, China[29]. The genome of strain JS14 consists of a single circular chromosome which is 2,137,435 bp in length, with a GC content of 41.22%. No plasmids were found and the chromosome has 2,106 CDSs. Comparative analyses revealed that strain ST1 GZ1 acquired 1AMR tet(W)), JS14 gained 6 AMRs (APH(3')-IIIa, tet(O), tet(40), SAT-4, aad(6), ErmB) and *S.suis* 366 harbored 2 AMRs (tet(O), ErmB).

Discussion and conclusion

Streptococcus suis, a zoonotic pathogen circulating in swine, can cause severe human infection and can lead to fatal complications, posing a serious public health threat worldwide[30]. MLST has been widely used to genetically classify bacterial strains. To date, ST1 has been predominantly reported to be responsible for human infections among invasive isolates worldwide[7]. However, few studies have investigated strains directly isolated from the cerebrospinal fluid of patients with purulent meningitis[31].

The results indicated that the isolated strain 366 was highly resistant to erythromycin, clindamycin and tetracycline. CARD identified tet(O) and ermB, which confer resistance to tetracycline and macrolide-lincosamide in the strain. Previous studies have also revealed that tet(O) and ermB are commonly found in *S. suis* strains from pigs and humans worldwide[32,33]. *Streptococcus suis* 366 was predicted to be highly pathogenic (the probability of being a human pathogen = 0.921 out of 1.0). Notably, virulence-associated genes, such as *mrp*, *sly*, *epf*, *ofs*, *revS*, *nadR*, *neuB*, and *neuC*, are typically found in highly pathogenic *S. suis* strains[18,34]. In the study, three genes related to capsule synthesis, *hasC*, *cpsF* and *neuB*, were identified. The product is a capsule, which is undisputedly considered the major virulence factor[35]. *S. suis* survival in blood and its dissemination depend on the production of a thick capsule, which protects the bacterium against immune recognition and immune clearance[36]. Protein PavA was the first Fibronectin-binding protein identified in *S. pneumoniae*, which acts directly as a fibronectin adhesin[37]. The presence of these 4 genes may contribute to the virulence of the isolate by potentially enhancing its ability to evade the immune system and adhere to host.

Antimicrobial resistance genes spread rapidly due to different mobile genetic elements (MGEs)[38]. The primary MGE categories are insertion sequences (IS), transposons (Tn), gene cassette/integron systems (integrons), plasmids, integrative and conjugative elements (ICE), and bacteriophages[39]. The main driving force behind spreading resistance between the environmental and clinical bacteria is horizontal gene transfer mediated by MGEs, such as transposons, plasmids and lysogenic bacteriophages[40]. In this study, there are 5 MGEs scattering across the genome, divided into 4 IS and 1 Tn. 3 MGEs belong to ISL3 family and 2 MGEs belong to IS110 family. However, Comprehensive genomic analysis revealed that the identified virulence and antimicrobial resistance genes were not located on known mobile genetic elements. The absence of key virulence and resistance genes from mobile genetic elements suggests a lower immediate risk of horizontal gene transfer among bacterial populations. Instead, the dissemination of these strains is likely driven by clonal expansion, which has implications for outbreak management and source tracking.

Prokaryotes have evolved a wide repertoire of defence systems to prevent invasion by mobile genetic elements (MGEs). However, because MGEs are vehicles for the exchange of beneficial accessory genes, defence systems

could consequently impede rapid adaptation in microbial populations[41]. CRISPR–Cas functions as a widespread adaptive immunity system that protects archaea and bacteria against viruses and other MGEs[42]. Analyses of the CRISPR–Cas system revealed one CRISPR array, 1 CRISPR array and two Cas clusters. The CRISPR structure may limit the horizontal transfer of drug resistance and virulence genes.

In this study, various databases and annotation tools were employed. Detailed analyses and interpretation of aspects such as the function and structure of each gene and their roles in the life activities of bacteria were carried out. The results of the GO analysis indicated that the genome was the most prominently enriched biological process category terms related to 'cellular process' and 'metabolic process', suggesting that the gene set is primarily involved in cellular adaptation. The most significantly enriched pathway was the 'ko02010 ABC transporter pathway', which may function as exporters that pump drugs across membranes. The results of COG analysis indicated that the functions of the genes were concentrated mainly in categories that were biologically significant for maintaining bacterial metabolism. Through these systematic and comprehensive gene annotations, the understanding of the *Streptococcus suis* ST1 genome has been further improved, laying a solid theoretical foundation for subsequent in-depth research on the biological characteristics and pathogenesis mechanisms of *Streptococcus suis*, as well as the development of targeted prevention and control strategies.

Phylogenetic analysis based on 16 S rRNA revealed that *Streptococcus suis* GZ1 is a close relative of the isolate characterized in this study. In a previous study, the highly pathogenic strain ST1 GZ1, which was isolated in 2005 from a patient in China who had septicemia, was representative of most strains isolated from humans in Europe and Asia[9]. Compared to *Streptococcus suis* GZ1, additional AMR gene ermB was found in *Streptococcus suis* 366. Furthermore, key virulence-associated factors (*hasC*, *cpsF*, *neuB*, and *pavA*) were identified in both strains. The SNP phylogeny provided a more resolved and accurate evolutionary relationship than the 16 S rRNA. The phylogenetic tree based on the core genome SNPs revealed *Streptococcus suis JS14* as the closest *Streptococcus suis* 366 relative. *S. suis* strain ST7 JS14 was isolated from articulation of a diseased pig in Jiangsu Province, China[29]. A recent study demonstrated that epidemic ST7 strains evolved from ST1 strains via a single-nucleotide change in the housekeeping gene thyA[9,10]. Phylogenetic analysis in this study revealed that ST1 and ST7 are closely related and that there may be an evolutionary connection. Pangenome analysis revealed that ST1 and ST7 share a large number of core genes, indicating their evolution during evolution.

In previous study, a strain of *S.suis* serotype 2 was isolated from a pig farm and subjected to whole-genome sequencing and characterization. The study have identified potential virulence genes associated with *S.suis* serotype 2, laying the foundation for further research on the virulence and resistance of *S.suis* serotype 2[43]. While our study employs the established methodological approach of genomic characterization with the previous published study, it is fundamentally distinguished by focusing on a clinically significant human ST1 isolate from purulent meningitis CSF. This direct analysis of a strain responsible for severe human disease provides unique insights into its pathogenesis. Furthermore, the inclusion of the strain from a new geographical region (Nanning) expands the diversity of sequenced isolates, offering a broader perspective on the pathogen's genetic landscape. Ultimately, our work significantly advances the understanding of *S.suis* by elucidating the genomic characteristics of a human-derived ST1 strain and clarifying its evolutionary relationship with the ST7 lineage.

A study conducted an investigation into the phylogenetic structure, genomic features, and virulence levels of 73 *S. suis* ST1 human strains from Guangxi between 2005 and 2020, which revealed significant diversity in phylogenetic structure and virulence levels among ST1 Guangxi strains[8]. while both studies employ genomic analyses of *S. suis* ST1, our work provides distinct and complementary insights by characterizing a recently isolated (2025) clinical strain, thereby offering a contemporary genetic snapshot critical for tracking this evolving pathogen. Furthermore, by incorporating strains from multiple regions and diverse hosts, our pan-genome and phylogenetic analyses significantly enhance the representativeness of evolutionary inferences. Transcending a purely comparative approach, our study delivers a functional genomic profile through systematic annotation (GO, KEGG, COG), revealing key enrichments in cellular/metabolic processes and ABC transporters, which advances the understanding of its genetic repertoire. Notably, we uncovered a substantial shared core gene set and affirmed a close evolutionary relationship between ST1 and ST7. Collectively, these findings establish a crucial foundation for elucidating the biological characteristics of *S. suis* ST1, ultimately informing targeted control strategies.

This study has several limitations that warrant consideration. These limitations include reliance on a single isolate, lack of functional validation of genomic predictions, constraints of comparative datasets, and the challenges in inferring virulence or transmission pathways solely from genomic data. Consequently, caution is advised when extrapolating the results. Future research should prioritize multi-isolate comparisons, experimental validation of key genomic features, and the integration of complementary datasets to reinforce causal inference. Accordingly, our subsequent work will employ functional genetics assays, including gene knockout and complementation experiments, to empirically determine the mechanistic role of the CRISPR system in the horizontal transfer of antibiotic resistance and virulence genes.

In conclusion, this study provides the detailed genomic characteristics associated with ST1 in *S.suis* and enhances the understanding of this zoonotic pathogen isolated from human CSF. Moreover, the results enhance the understanding of the evolution connection of the ST1 and ST7 strains.

Methods

Patient with purulent meningitis and bacterial isolation

The clinical strain *Streptococcus suis* 366 was isolated from the cerebrospinal fluid of a patient with purulent meningitis in the emergency intensive care unit. The patient demonstrated partial improvement in cognitive function following targeted antimicrobial therapy. However, severe sensorineural hearing loss was observed. The cultured isolate was identified by a matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) system (with the IVD database version 3.0, Bruker, Germany).

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined using the BD Phoenix™ M50 Automated Microbiology System (Becton, Dickinson and Company, USA). Ten types of commonly used drugs were selected, namely, aminoglycosides, cephems, glycopeptides, fluoroquinolones, oxazolidinones, carbapenems, phenicols, macrolides, lincosamides, and tetracyclines. *Streptococcus pneumoniae* ATCC 49619 was used as a quality control strain. The broth microdilution method was carried out in strict accordance with the current Clinical and Laboratory Standards Institute (CLSI) performance standards (M100-Ed35, 2025)[44], and the breakpoints for *β-haemolytic streptococci* were used.

DNA extraction and whole-genome sequencing

The bacterial genomic DNA was extracted using a bacterial genomic DNA isolation kit (Sangon Biotech, Shanghai, China). Whole-genome sequencing was subsequently conducted on the Illumina NovaSeq 6000 platform. Raw sequencing reads were processed through quality control analysis using FastQC[45], followed by adapter trimming and quality filtering with Trimmomatic[46]. De novo genome assembly was ultimately performed using the SPAdes[47] genome assembler (v3.5.0) with default parameters, and the assembled genome was annotated using the NCBI Prokaryote Genome Annotation Pipeline[48]. Busco V6.0.0 was used to assess the assembled bacterial genome[12].

Genome annotation

Multilocus sequence typing (MLST) was performed with MLST 2.0 using default parameters to determine the strain lineage[49]. CRISPR–Cas systems were annotated using CRISPRCasFinder to assess adaptive immunity elements[50]. The presence of plasmids was analysed using PlasmidFinder 2.1[51]. Circular *Streptococcus suis* 366 genome visualization was performed using Prokesee[52]. The mobile genetic elements in the genome were detected using MobileElementFinder[13]. The computational screening of antimicrobial resistance (AMR) genes was conducted through the Comprehensive Antibiotic Resistance Database using rigorous identity and coverage thresholds (≥ 90%)[53]. Virulence-associated genes were identified by alignment against the Virulence Factor Database (VFDB)[54], with a sequence identity threshold of 70%. Pathogenic potential was predicted using PathogenFinder, which evaluates host-specific pathogenicity based on genomic signatures[55]. Functional annotations, including Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and Clusters of Orthologous Groups of proteins (COG) analyses, were conducted for *Streptococcus suis* 366[56–58].

16 S rRNA-based phylogenetic analysis

The 16 S rRNA sequence of *Streptococcus suis* 366 was aligned to the NCBI database using BLAST with the parameter identity > 95%. Afterwards, the selection criteria for 16 S rRNA sequences included the following: (1) belonging to sequence type 1 (ST1); (2) being isolated from cerebrospinal fluid; (3) being isolated from infected pigs or human. Then, a phylogenetic tree was constructed using FastTree[59] after multiple sequence alignment and clipping using MAFFT[60].

Pangenome construction

The Roary program[61] was used to construct the pangenome with a minimum percentage identity of 95%. The common genes and unique genes of *Streptococcus suis* 366 and 7 related strains were obtained and analysed in depth. Functional annotation was performed by alignment with the Cluster of Orthologous Groups of proteins (COG) database to analyse and infer gene functions.

SNP-based phylogenetic analysis

Based on the results of whole-genome SNP analyses, a neighbour-joining phylogenetic tree was constructed with FastTree[59] incorporating 7 closely related reference strains (≥ 95% average nucleotide identity). The resultant phylogenetic topology was subsequently visualized using the Interactive Tree of Life (iTOL) web platform[62].

Genome comparisons with the closest pathogenic strains

In addition, genome comparisons were undertaken between the three genomes of the ST1 highly virulent strain *S. suis* GZ1, ST7 epidemic *S. suis* JS14, and the study strain *S. suis* 366, using the Mauve software and following the instructions[63].

Data availability

All original data used and analysed during the current study are available from the corresponding author upon reasonable request. The whole-genome shotgun sequence of *Streptococcus suis* 366 has been deposited in GenBank under the accession JBLMKP000000000.

Received: 4 June 2025; Accepted: 20 February 2026
Published online: 27 February 2026

References

1. Goyette-Desjardins, G., Auger, J.-P., Xu, J., Segura, M. & Gottschalk, M. *Streptococcus suis*, an important pig pathogen and emerging zoonotic agent—an update on the worldwide distribution based on serotyping and sequence typing. *Emerg. Microbes Infect.* **3**, e45 (2014).
2. Higgins, R. & Gottschalk, M. Streptococcal diseases. In *Diseases of Swine*, 9th edn. (eds. Straw, B.E. et al.) 769–783 (University of Iowa Press, Iowa City, IA, 2005).

3. Bonifait, L., Veillette, M., Létourneau, V., Grenier, D. & Duchaine, C. Detection of *streptococcus suis* in bioaerosols of swine confinement buildings. *Appl. Environ. Microbiol.* **80**, 3296–3304 (2014).
4. Lv, R. et al. Current prevalence and therapeutic strategies for porcine *streptococcus suis* in China. *Appl. Environ. Microbiol.* **91**, e02160-24 (2025).
5. Liang, S. et al. The ABC type fucose operon regulated by XtrSs through CcpA contributes to *streptococcus suis* survival in macrophages. *Virulence* **16**, 2553790 (2025).
6. Lun, Z.-R., Wang, Q.-P., Chen, X.-G., Li, A.-X. & Zhu, X.-Q. *Streptococcus suis*: An emerging zoonotic pathogen. *Lancet Infect. Dis.* **7**, 201–209 (2007).
7. Huong, V. T. L. et al. Epidemiology, clinical manifestations, and outcomes of *streptococcus suis* infection in humans. *Emerg Infect. Dis* **20**, (2014).
8. Kang, W. et al. Investigation of genomic and pathogenicity characteristics of *streptococcus suis* ST1 human strains from guangxi zhuang autonomous region (GX) between 2005 and 2020 in China. *Emerg. Microbes Infect.* **13**, 2339946 (2024).
9. Ye, C. et al. Clinical, experimental, and genomic differences between intermediately pathogenic, highly pathogenic, and epidemic *streptococcus suis*. *J. Infect. Dis.* **199**, 97–107 (2009).
10. Wang, M. et al. Genomic epidemiology of *streptococcus suis* sequence type 7 sporadic infections in the Guangxi Zhuang Autonomous Region of China. *Pathogens* **8**, 187 (2019).
11. Murray, G. G. R. et al. The emergence and diversification of a zoonotic pathogen from within the microbiota of intensively farmed pigs. *Proc. Natl. Acad. Sci. U. S. A.* **120**, e2307773120 (2023).
12. Tegenfeldt, F. et al. OrthoDB and BUSCO update: Annotation of orthologs with wider sampling of genomes. *Nucleic Acids Res.* **53**, D516–D522 (2024).
13. Johansson, M. H. K. et al. Detection of mobile genetic elements associated with antibiotic resistance in *salmonella enterica* using a newly developed web tool: MobileElementFinder. *J. Antimicrob. Chemother.* **76**, 101–109 (2020).
14. Alcock, B. P. et al. CARD 2023: Expanded curation, support for machine learning, and resistome prediction at the comprehensive antibiotic resistance database. *Nucleic Acids Res.* **51**, D690–D699 (2022).
15. Weisblum, B. Erythromycin resistance by ribosome modification. *Antimicrob. Agents Chemother.* **39**, 577–585 (1995).
16. Leclercq, R. Mechanisms of resistance to macrolides and lincosamides: Nature of the resistance elements and their clinical implications. *Clin. Infect. Dis.* **34**, 482–492 (2002).
17. Li, W. et al. Mechanism of tetracycline resistance by ribosomal protection protein tet(O). *Nat. Commun.* **4**, 1477 (2013).
18. Zheng, X. et al. Identification of genes and genomic islands correlated with high pathogenicity in *streptococcus suis* using whole genome tilling microarrays. *PLoS ONE* **6**, e17987 (2011).
19. Gottschalk, M., Segura, M. & Xu, J. *Streptococcus suis* infections in humans: The chinese experience and the situation in North America. *Anim. Health Res. Rev.* **8**, 29–45 (2007).
20. Smith, H. E. et al. Identification and characterization of the cps locus of streptococcus suis serotype 2: The capsule protects against phagocytosis and is an important virulence factor. *Infect. Immun.* **67**, 1750–1756 (1999).
21. Charland, N., Kobisch, M., Martineau-Doizé, B., Jacques, M. & Gottschalk, M. Role of capsular sialic acid in virulence and resistance to phagocytosis of *streptococcus suis* capsular type 2. *FEMS Immunol. Med. Microbiol.* **14**, 195–203 (1996).
22. Ashburner, M. et al. Gene ontology: Tool for the unification of biology. *Nat. Genet.* **25**, 25–29 (2000).
23. Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M. & Tanabe, M. KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Res.* **44**, D457–D462 (2016).
24. Tomii, K. & Kanehisa, M. A comparative analysis of ABC transporters in complete microbial genomes. *Genome Res.* **8**, 1048–1059 (1998).
25. Rees, D. C., Johnson, E. & Lewinson, O. ABC transporters: The power to change. *Nat. Rev. Mol. Cell Biol.* **10**, 218–227 (2009).
26. Beis, K. Structural basis for the mechanism of ABC transporters. *Biochem. Soc. Trans.* **43**, 889–893 (2015).
27. Tatusov, R. L., Galperin, M. Y., Natale, D. A. & Koonin, E. V. The COG database: A tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Res.* **28**, 33–36 (2000).
28. Ferraz Helene, L. C., Klepa, M. S. & Hungria, M. New insights into the taxonomy of bacteria in the genomic era and a case study with rhizobia. *Int. J. Microbiol.* **2022**, 4623713 (2022).
29. Hu, P. et al. Complete genome sequence of *streptococcus suis* serotype 14 strain JS14. *J. Bacteriol* **193**, 2375–2376 (2011).
30. Brizuela, J. et al. Molecular epidemiology of underreported emerging zoonotic pathogen *streptococcus suis* in Europe. *Emerg. Infect. Dis.* **30**, 413–422 (2024).
31. Liu, F. et al. *Streptococcus suis*: Epidemiology and resistance evolution of an emerging zoonotic bacteria. *One Health* **21**, 101098 (2025).
32. Yongkiettrakul, S. et al. Genome sequences of antibiotic-resistant *streptococcus suis* strains isolated from human patients and diseased and asymptomatic pigs in Thailand. *Infect. Genet. Evol.* **87**, 104674 (2021).
33. Kerdsin, A. et al. Genomic characterization of *streptococcus suis* serotype 24 clonal complex 221/234 from human patients. *Front. Microbiol.* **12**, 812436 (2021).
34. Huang, W. et al. Genomic epidemiological investigation of a *streptococcus suis* outbreak in Guangxi, China, 2016. *Infect. Genet. Evol.* **68**, 249–252 (2019).
35. Segura, M., Fittipaldi, N., Calzas, C. & Gottschalk, M. Critical *streptococcus suis* virulence factors: Are they all really critical?. *Trends Microbiol.* **25**, 585–599 (2017).
36. Fittipaldi, N., Segura, M., Grenier, D. & Gottschalk, M. Virulence factors involved in the pathogenesis of the infection caused by the swine pathogen and zoonotic agent *streptococcus suis*. *Future Microbiol.* **7**, 259–279 (2012).
37. Kadioglu, A. et al. Pneumococcal protein PavA is important for nasopharyngeal carriage and development of sepsis. *Mol. Oral Microbiol.* **25**, 50–60 (2010).
38. Cross, B. J., Partridge, S. R. & Sheppard, A. E. Impacts of mobile genetic elements on antimicrobial resistance genes in gram-negative pathogens: current insights and genomic approaches. *Microbiol. Res.* **302**, 128340 (2026).
39. Partridge, S. R., Kwong, S. M., Firth, N. & Jensen, S. O. Mobile genetic elements associated with antimicrobial resistance. *Clin. Microbiol. Rev.* **31**, e00088–e00017 (2018).
40. Pärnänen, K. et al. Evaluating the mobility potential of antibiotic resistance genes in environmental resistomes without metagenomics. *Sci. Rep.* **6**, 35790 (2016).
41. Liu, Y., Botelho, J. & Iranzo, J. Timescale and genetic linkage explain the variable impact of defense systems on horizontal gene transfer. *Genome Res.* **35**, 268–278 (2025).
42. Watson, B. N. J. et al. CRISPR-cas in pseudomonas aeruginosa provides transient population-level immunity against high phage exposures. *ISME J.* **18**, wrad039 (2024).
43. Zhou, Y. et al. Pan-genome analysis of streptococcus suis serotype 2 highlights genes associated with virulence and antibiotic resistance. *Front. Microbiol.* **15**, 1362316 (2024).
44. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing–35nd Edition. M100. Wayne, PA. (2025).
45. Andrews, S. & FastQC A quality control tool for high throughput sequence data. *Babraham Bioinf.* (2010). https://www.bioinformatics.babraham.ac.uk/projects/fastqc/(
46. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: A flexible trimmer for illumina sequence data. *Bioinformatics* **30**, 2114–2120 (2014).

47. Bankevich, A. et al. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* **19**, 455–477 (2012).
48. Tatusova, T. et al. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res.* **44**, 6614–6624 (2016).
49. Larsen, M. V. et al. Multilocus sequence typing of total-genome-sequenced bacteria. *J. Clin. Microbiol.* **50**, 1355–1361 (2012).
50. Couvin, D. et al. CRISPRCasFinder, an update of CRISRFinder, includes a portable version, enhanced performance and integrates search for cas proteins. *Nucleic Acids Res.* **46**, W246–W251 (2018).
51. Carattoli, A. et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob. Agents Chemother.* **58**, 3895–3903 (2014).
52. Grant, J. R. et al. Proksee: In-depth characterization and visualization of bacterial genomes. *Nucleic Acids Res.* **51**, W484–W492 (2023).
53. McArthur, A. G. et al. The comprehensive antibiotic resistance database. *Antimicrob. Agents Chemother.* **57**, 3348–3357 (2013).
54. Chen, L. et al. VFDB: A reference database for bacterial virulence factors. *Nucleic Acids Res.* **33**, D325–D328 (2005).
55. Cosentino, S., Voldby Larsen, M., Møller Aarestrup, F. & Lund, O. PathogenFinder - Distinguishing friend from foe using bacterial whole genome sequence data. *PLoS ONE* **8**, e77302 (2013).
56. The gene ontology. resource: enriching a GOld mine. *Nucleic Acids Res.* **49**, D325–D334 (2020).
57. Ogata, H. et al. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **27**, 29–34 (1999).
58. Tatusov, R. L., Galperin, M. Y., Natale, D. A. & Koonin, E. V. The COG database: A tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Res.* **28**, 33–36 (2000).
59. Price, M. N., Dehal, P. S. & Arkin, A. P. FastTree: Computing large minimum evolution trees with profiles instead of a distance matrix. *Mol. Biol. Evol.* **26**, 1641–1650 (2009).
60. Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **30**, 772–780 (2013).
61. Page, A. J. et al. Roary: Rapid large-scale prokaryote pan genome analysis. *Bioinformatics* **31**, 3691–3693 (2015).
62. Letunic, I. & Bork, P. Interactive tree of life (iTOL) v6: Recent updates to the phylogenetic tree display and annotation tool. *Nucleic Acids Res.* **52**, W78–W82 (2024).
63. Darling, A. C. E., Mau, B., Blattner, F. R. & Perna, N. T. Mauve: Multiple alignment of conserved genomic sequence with rearrangements. *Genome Res.* **14**, 1394–1403 (2004).

Author contributions
All the authors contributed to the study conception and design. JJ reviewed the literature, analysed the patient data and wrote the first draft of the paper. WD analysed the patient data and collected the data. LL reviewed the first draft of the paper and edited it. All the authors commented on previous versions of the manuscript. All the authors have read and approved the final manuscript for publication.

Funding
This work was supported by the Guangxi Natural Science Foundation [2023GXNSFBA026028].

Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
This research complies with the guidelines for human studies and is in accordance with the Declaration of Helsinki. This report was approved by the Medical Ethics Committee of the People's Hospital of Guangxi Zhuang Autonomous Region (ethical approval no. KY-KJT-2023-149).

Consent for publication
Written informed consent was obtained from the patient.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-026-41475-6.

Correspondence and requests for materials should be addressed to L.L.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

© The Author(s) 2026