Heliyon 10 (2024) e35456

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Identification and genomic analyses of a *Streptococcus suis* ST25 strain associated with the first human septicemia in mainland China

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ARTICLE INFO

Keywords:
Streptococcus suis
ST25
Case reports
Genome analysis
Gene annotation

ABSTRACT

Streptococcus suis (*S. suis*) is a Gram-positive bacterium and the main culprit behind zoonotic outbreaks, posing a serious threat to public health. The prevalent strains in China are mainly of sequence types (ST) 1 and 7, with few cases of human infections caused by other sequence type being reported. This study presents the first isolation of a ST25 strain from the blood of a septicemic patient. A 57-year-old febrile patient was admitted to a hospital in Hainan of China, diagnosed as septicemia and hepatic dysfunction. A strain of *S. suis* was isolated from blood culture and confirmed to be serotype 2 and ST25 through 16S rRNA sequencing and whole-genome sequencing, and its genome was further analyzed for gene functions and presence of drug resistance genes. The full-length genome of strain HN28 spans 2,280,124 bp and encodes a total of 2291 proteins. Genes annotated in COG, GO, KEGG, CAZy, and PHI databases accounted for 75.38 %, 69.14 %, 55.35 %, 4.58 %, and 11.87 % of the total predicted proteins, respectively. Virulence factor analysis revealed the presence of seven putative virulence genes in strain HN28. Analysis using the CARD database identified 51 resistance genes in HN28, alongside abundant exocytosis systems. These findings underscore the occurrence of *S. suis* infections in humans caused by less common ST, emphasizing the need for enhanced epidemiological investigations and monitoring of *S. suis* infections in the human population.

1. Introduction

Streptococcus suis (*S. suis*) has become a significant zoonotic pathogen in countries with a substantial pig industry [1], with pigs

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https://doi.org/10.1016/j.heliyon.2024.e35456
Received 26 March 2024; Received in revised form 21 July 2024; Accepted 29 July 2024
Available online 2 August 2024

serving as the primary hosts, and human infections essentially occurring through close contact with diseased pigs and raw pork [2,3]. Infections with this Gram-positive coccobacillus have been presented as various diseases, including arthritis, endocarditis, meningitis, sepsis, toxic shock syndrome, and even death [4]. The diseases caused by this pathogen pose substantial economic burden on the pig industry and represent a potential life threat to pigs in particular and individuals in close contact, such as pig farmers, slaughterhouse workers, and transportation personnel [5]. Since the first reported human case in Denmark in 1968, thousands of human cases of *S. suis* infection have been reported globally, with the majority occurring in Asian countries [6]. The 2005 *S. suis* infection outbreak in Sichuan Province of China resulted in over 200 human cases and heavy fatalities [7,8], drawing widespread attention.

One of the conventional methods for typing *S. suis* is based on the antigenic specificity of capsular polysaccharides (*cps*), and 29 serotypes of *S. suis* have so far been described, with serotype 2 being the most common and pathogenic in both humans and diseased pigs [9,10]. However, the serotype of an isolated strain is not the decisive factor for virulence [11], and heterogeneity has been observed in human cases of serotype 2 infections, suggesting that individual serotyping may not fully represent the diversity of *S suis* strains [12]. Additionally, multilocus sequence typing (MLST) based on nucleotide sequences is also the other commonly used typing scheme in epidemiological investigations [13]. As of July 8 of 2024, a total of 4111 allelic gene sequences have been registered. Among them, ST1 is the most widely distributed and numerous ST, primarily found in Europe and Asia, with ST7 only being isolated in China so far [14]. In North American countries, the most common types are ST25 and ST28, which are frequently isolated from diseased pigs [15].

Currently, high-throughput and high-coverage sequencing holds the promise for widespread use in translational medicine. Data from whole-genome sequencing (WGS) may be utilized for the identification and classification of micro-organisms [16], which has been extensively employed in the study of *S. suis* [11,17–19]. Here, we present the clinical characteristics of a septicemic patient, and the genomic features, typing, gene functions, as well as the virulence factors and drug resistance gene profile of a ST25 *S. suis* strain (HN28) isolated from the blood of the patient.

2. Materials and methods

2.1. Bacterial isolation and identification

The patient's blood specimen was streaked onto Columbia blood agar plates and incubated for 20 h in an incubator at 37 °C with 5 % CO_2. A fresh colony was selected for Gram staining and microscopy, and similar colonies on the same plate were chosen for PCR amplification. Primers (5′-AGAGTTTGATCMTGGCTCAG-3'; 5′-GGYTACCTTGTTACGACTT-3′) were designed as described [20], PCR products were run through 1.2 % agarose gel electrophoresis, and subjected to Sanger sequencing. Following purification and cultivation, the strain (HN28) was further cultured, purified, preserved in a 30 % glycerol solution and stored at −80 °C.

2.2. Detection of virulence-related genes

Total genomic DNA was extracted according to the manufacturer's instructions (TIANamp Bacteria DNA Kit) from fresh culture of HN28 strain. Using primers detailed in Table S1 and a described procedure [11], PCR amplification was then performed to detect the presence of virulence-related genes, including extracellular protein factor (*ef*), suilysin (*sly*), muramidase released protein (*mrp*), capsular polysaccharide 2 J (*cps2J*), open reading frame 2 (*orf2*), glutamate dehydrogenase (*gdh*), fibronectin-binding protein (*fbps*), and glyceraldehyde 3-phosphate dehydrogenase (*gapdh*). Negative controls used nuclease-free water as the template.

2.3. Antimicrobial susceptibility profiles

Following the methods outlined in the Clinical and Laboratory Standards Institute (CLSI) guidelines [21], a sterile cotton swab was used to uniformly spread on Columbia blood agar plates the HN28 suspension adjusted to 0.5 McFarland standard (1.5×10^8 colony-forming units/mL). Individual sterile forceps were used to place different antibiotic discs on the agar, and each agar plate with four different antibiotics well separated. The plates were then incubated for 20 h at 37 °C and supplied with 5 % CO_2. *Staphylococcus aureus* (ATCC 25923) served as the quality control, and all procedures were conducted in a sterile environment. The Kirby-Bauer method [22,23] was employed to assess the susceptibility of strain HN28 to 12 antibiotics, including cefpirome and levofloxacin.

2.4. Whole-genome sequencing

Total genome DNA was extracted as described in 2.2 and sequenced using the Illumina HiSeq TM2000 sequencing platform by Beijing Nuohe Zhiyuan Biotech Co., Ltd. WGS analysis of the HN28 genome was conducted by filtering the raw sequencing data and the assembly was performed using the SOAP software. GeneMarkS software (*http://topaz.gatech.edu/*) was employed to predict open reading frames (ORFs), and bacterial coding gene prediction was conducted by comparing the predicted ORFs with non-redundant protein databases (KEGG and COG, among others). Gene island content was predicted using IslandPath-DIOMB software, and tRNAs and rRNAs were predicted using tRNA scan-SE software and Rfam software, respectively. The ST of the isolated strain was analyzed using PubMLST (*https://pubmlst.org/*) [13]. Virulence factor prediction was achieved through the virulence factor database (VFDB, *http://www.mgc.ac.cn/VFs/*), and resistance genes were predicted using the comprehensive antibiotic resistance database (CARD, *https://card.mcmaster.ca/*).

2.5. Phylogenetic analysis

The geoBURST algorithm in the Phyloviz software (*http://www.phyloviz.net/*) was employed for ST analysis [24]. Based on a prior research, genomes of 11 ST25 strains were selected and the complete genomes of representative ST1 and ST7 strains from domestic sources were also included for comparative purposes. Single nucleotide polymorphisms (SNPs) were detected, using the NSUI060 genome sequence as a reference [25] and the MUMmer software to select mutation SNP sites. All bacterial genome data were sourced from the NCBI database (*https://www.ncbi.nlm.nih.gov/*, Table S2), the maximum likelihood method was employed to construct the phylogenetic tree, with *Streptococcus pneumoniae* (ATCC 700669) used as outgroup to root the tree, and the resulting tree was further enhanced using TVBOT (*https://www.chiplot.online/*) for visualization.

3. Results

3.1. Case introduction

In June 2023, a middle-aged male was admitted to a third-tier Hainan hospital with symptoms of fever, dizziness, headache, chest tightness, and shortness of breath after exposure to cold. The patient worked in raw pork retail just before coming to the hospital, had a history of left cerebellar infarction diagnosed two years ago, and with a stage 3 hypertension voluntarily discontinued medication after discharge, but denied any history of blood transfusion, exposure to contaminated water, or chronic diseases such as diabetes and infectious diseases. Upon admission, blood cell analysis, high-sensitivity CRP, and initial CT scans of the head were conducted due to consideration of possible intracranial infection. Subsequently, blood culture tests were performed, and a final diagnosis of septicemia was made and supported by the isolation of pure *S. suis* bacteria from the blood culture of the patient, and his raw pork contact history. He asked to be discharged after seeing the improved condition upon treatment. Some of his clinical characteristics and laboratory test results conducted after admission are presented in Table 1.

3.2. Isolation and characterization of S. suis

Pure bacteria were isolated from the blood of the above patient hospitalized in the infectious disease ward and a single purified colony was reinoculated onto Columbia blood agar plates and cultured for 20 h at 37 °C. Observed were smooth, moist, translucent colonies with distinct α-hemolysis halos around them (Fig. 1), and the bacterium was subsequently confirmed as *S. suis* (hereafter strain HN28) by PCR and Sanger sequencing.

3.3. Molecular typing of S. suis

3.3.1. Serological typing

All types of *cps* are believed to be synthesized through the *wzx/wzy* pathway [26], and the *wzy* gene was found to be the determination gene for serotypes of *S. suis* upon analysis of its *cps* gene cluster sequences [27]. By aligning its genome sequence with the *wzy* sequence, strain HN28 was identified to be serotype (SS) 2.

3.3.2. MLST typing

Strain HN28 was identified as ST25 (Table 2) according to its seven housekeeping gene profile [13] and by using the designated PubMLST website (https://pubmlst.org/organisms/) for *S. suis*. ST25 belongs to the clone initiation in the CC25 complex as revealed by the goeBURST analysis (Fig. 2), and is a subgroup creator of the CC28 complex along with CC117, CC27, CC142, CC29, CC25, and CC380.

3.3.3. Virulence factor characterization

The presence of virulence factor genes (*cps2J*, *orf2*, *gdh*, *fbps*, and *gapdh*) in strain HN28 was demonstrated by PCR, with the notable absence of some classic virulence genes (*ef*, *sly*, and *mrp*) (Fig. 3).

Table 1
Some of the clinical characteristics of the patient infected with *Streptococcus suis* HN28.

Parameters	Results
Age	57 years
Sex	Male
Job	Pork retailing
Temperature	39.2 °C
Pulse rate	82 beats/minute
Respiratory rate	20 breaths/minute
Blood pressure	140/84 mmHg
CRP	144.04 mg/mL
Outcome	Recovered

Fig. 1. Gross colony morphology of *Streptococcus suis* strain HN28 on the Columbia blood agar plate. When cultured for 24 h, the HN28 colonies were grayish white, round, smooth, translucent, moist, and surrounded by α-hemolytic halos.

Table 2
Sequence type of strain HN28.

Strain	*aroA*	*cpn60*	*dpr*	*gki*	*mutS*	*recA*	*thrA*	ST
HN28	9	30	5	34	30	3	25	25

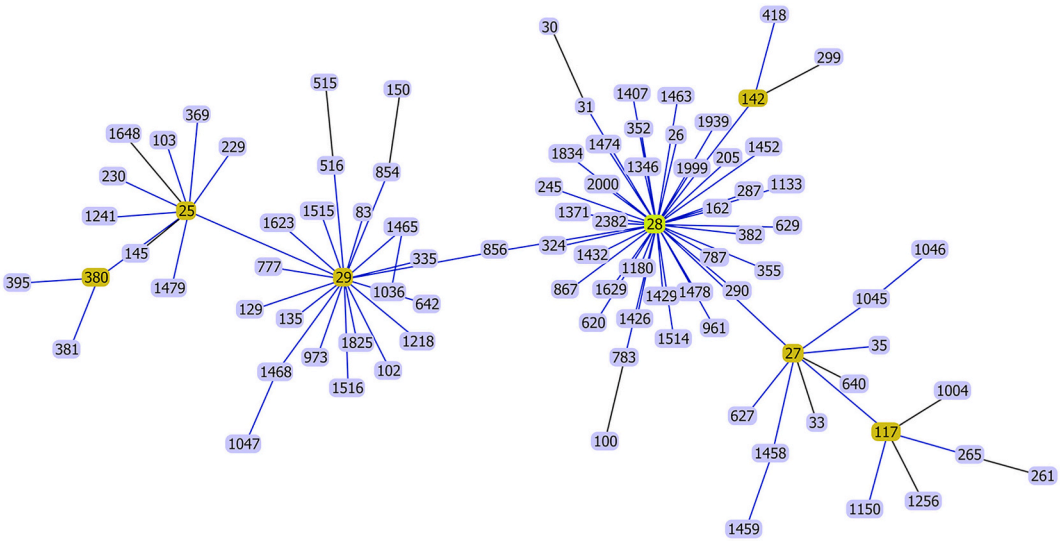

Fig. 2. GoeBURST analysis of ST25 (yellow color) and other *Streptococcus suis* sequence types (centered around ST27, ST28, ST29, ST117, ST142 and ST380) by Phyloviz software available online at https://pubmlst.org/. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.4. Basic genomic characterization

As highlighted in Table 4, the size of the HN28 genome (GC content, 40.83 %) is 2,280,124 bp, with a total of 2291 genes (an average gene length of 867 bp, total gene length of 1,986,243 bp, and a gene coding ratio of 87.11 %), encoding 2291 proteins, 62

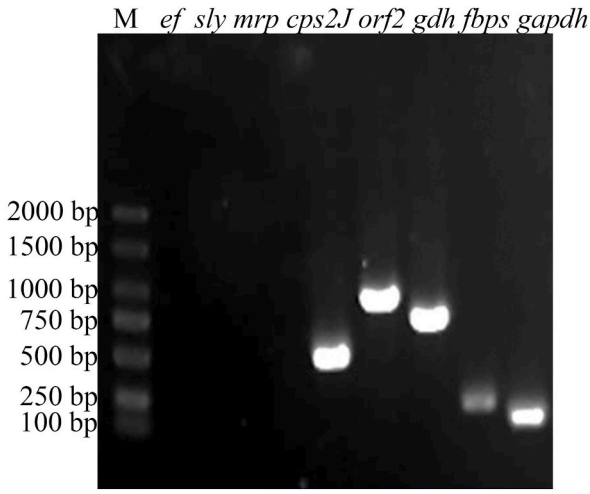

Fig. 3. The eight individually PCR-amplified virulence factor gene products (*ef*, *sly*, *mrp*, *cps2J*, *orf2*, *gdh*, *fbps*, and *gapdh;* lanes 2–9) of strain HN28 were subjected to 1.2 % agarose gel electrophoresis, and the DNA sizes of the respective product were estimated according to the DNA ladder (M, lane 1).

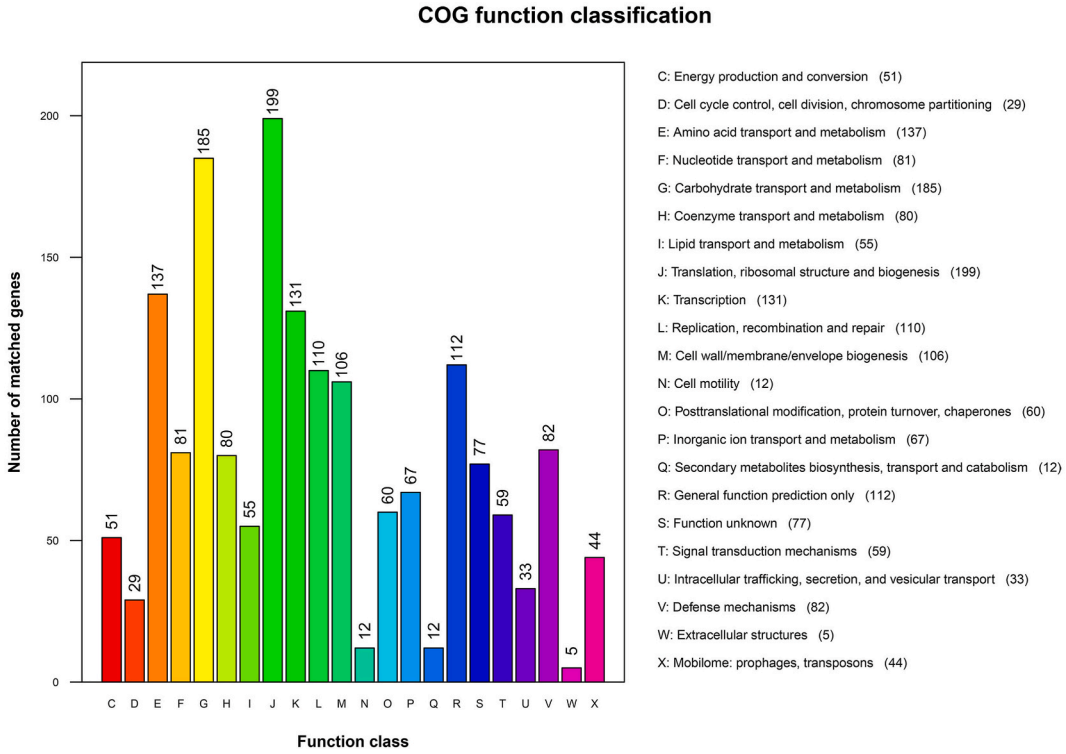

Fig. 4. The genome of *Streptococcus suis* strain HN28 was functionally classified by the Cluster of Orthologous Groups (COG) into different COG functional classes and plotted into gradient color bars, the number of genes involved in each functional class was shown at the top of the bar and at the end of the functional class category represented by each single-letter code (C–X). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

tRNAs, and 4 sRNAs. The assembly consists of 81 scaffolds, and the prediction using IslandPath-DIOMB software identified 5 genomic islands.

3.5. Genome annotation

3.5.1. Annotation results from the COG database

COG is a protein cluster database of orthologous groups created and maintained by the NCBI. Alignment analysis using the COG database reveals that strain HN28 has 1727 functional genes that can be classified into 22 COG subcategories, accounting for 75.38 % of the total proteins. The most abundant ones include categories associated with translation, ribosomal structure and biogenesis (199, 11.52 %), followed by carbohydrate transport and metabolism (185, 10.71 %), amino acid transport and metabolism (137, 7.93 %), transcription (131, 7.59 %), and others (Fig. 4). In summary, majority of the proteins encoded by the HN28 genome are primarily involved in translation, ribosomal structure and biogenesis, carbohydrate transport and metabolism, as well as transcription, a pattern quite similar to that of 19 genomes belonging to the other four ST (ST1, 7, 19, 28) types [28].

3.5.2. Annotation results from the GO database

The GO database is a classification system that encompasses three major functions: cellular components, molecular functions, and biological processes (detailed in Fig. 5). The 1584 predicted proteins of strain HN28 are annotated by the GO database, accounting for 69.14 % of the total proteins. Among them, the top 5 categories are catalytic activity and binding (two of the 10 categories of molecular functions), cellular anatomical entity (one of the 5 categories of cellular components), and metabolic process and cellular process (two of the 20 categories of biological processes). In summary, the main molecular functions of HN28 proteins are predicted to be involved in catalysis and binding, cellular anatomical entity, and cellular/metabolic processes.

3.5.3. Annotation results from the KEGG database

KEGG is a comprehensive database for the study of metabolic pathways, including genomic information, chemical information, system functional information, as well as information on diseases and health. Only 1268 genes of strain HN28 are mapped, accounting for 55.35 % of the total proteins. These genes (Fig. 6) are primarily associated with carbohydrate metabolism pathways (288), membrane transport pathways (151), and amino acid metabolism pathways (93). In summary, KEGG analysis highlights that pathways related to amino acid/carbohydrate metabolism and membrane transport appear to be critical for strain HN28.

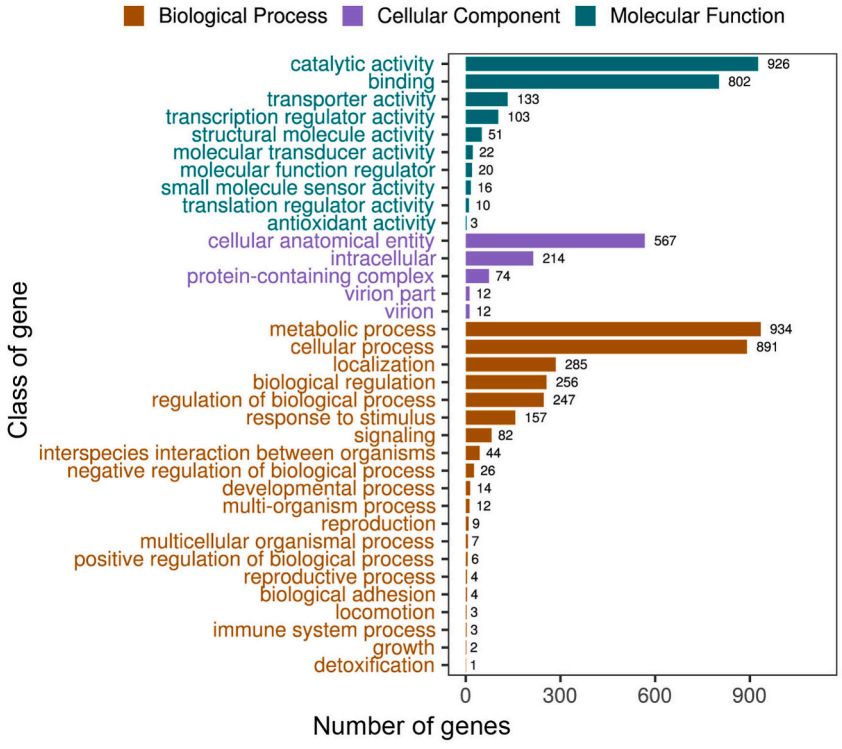

Fig. 5. Functional classification of genome of *Streptococcus suis* strain HN28 was predicted by the Gene Ontology (GO), showing how many gene(s) involved in each sub-category of the three main categories: biological processes, cellular components, and molecular functions.

Fig. 6. Functional classification of genome of *Streptococcus suis* strain HN28 as annotated by the Kyoto Encyclopedia of Genes and Genomes (KEGG), showing how many gene(s) involved in each sub-pathway of the six categories.

3.5.4. Annotation results from the CAZy database

CAZy is a carbohydrate enzyme-related database, comprising five main classes and one structural domain. Using the Diamond software to align the amino acid sequences of strain HN28 with the CAZy database, 51 genes are for glycoside hydrolases, 37 genes for glycosyltransferases, 12 genes for carbohydrate-binding modules, 3 genes for carbohydrate esterases, 2 genes for polysaccharide lyases, and none for auxiliary activities. In total, 105 carbohydrate-active enzyme genes are annotated in the HN28 genome, accounting for 4.58 % of the total genes, suggesting its capability to utilize various sugars may help the bacterium adapt well to challenging environments.

Table 3
Drug sensitivity experiment results.

Drug name	Drug content (μg/tablet)	Judgment criteria			Diameter (mm)	Results
		R	I	S		
Azithromycin	15	≤13	14–17	≥18	0	R
Clindamycin	2	≤15	16–18	≥19	0	R
Erythromycin	15	≤15	16–20	≥21	0	R
Kanamycin	30	≤13	14–17	≥18	0	R
Streptomycin	10	≤11	12–14	≥15	0	R
Tetracycline	30	≤18	19–22	≥23	15	R
Cefepime	30	≤21	22–23	≥24	26	S
Chloramphenicol	30	≤17	18–20	≥21	22	S
Gatifloxacin	5	≤17	18–20	≥21	23	S
Levofloxacin	5	≤13	14–16	≥17	20	S
Meropenem	10	≤19	20–22	≥23	27	S
Sulfamethoxazole/trimethoprim	25	≤10	11–15	≥16	20	S

Note: R: Resistant; I: Intermediary; S: Sensitive.

3.5.5. Annotation results from the PHI database

PHI is a gene database that focuses on the interaction between pathogens and hosts. A total of 272 genes in the HN28 genome are annotated by this database, accounting for 11.87 % of the total genes. When classified based on phenotype, 177 genes are supposed to reduce virulence, 53 genes have no effect on pathogenicity, 17 genes are to enhance virulence (hyper-virulence), 14 genes are labeled as lethal, 9 genes are to result in loss of pathogenicity, and one gene each annotated for chemical sensitivity and drug resistance. In summary, genes predicted to modulate virulence and those unannotated or having yet unknown functions need to be experimentally tested in further exploration and investigation.

3.5.6. Virulence factor analysis from PCR and VFDB database

WGS once again confirms our PCR results that HN28 does not carry genes encoding extracellular protein factor (*ef*), suilysin (*sly*), muramidase released protein (*mrp*), respectively (Table S3). It does have other genes encoding putative virulence factors that may play roles in binding, adherence, invasion, and immune evasion. Following are the putative virulence factor candidates (Table S3) predicted by the VFDB database that remain to be experimentally verified, the agglutinin receptor encoded by *orf01482* and plasmin receptor/GAPDH encoded by *plr/gapA* (orf00040), the choline-binding protein encoded by *cbpD* (orf00468) and fibronectin-binding protein encoded by *pavA* (orf01597), the enolase encoded by *orf01604*, trigger factor encoded by *tig/ropA* (orf00768), and the capsule encoded by its 18 *orfs* (orf00911, orf01060-01070; orf01074-01079).

3.5.7. Antibiotic profile tested and resistance genes predicted by CARD database

According to the reported *S. suis* antibiotic resistance genes [2,29], 12 antibiotics were tested three times on strain HN28 for its resistance profile with identical results. Initial screening (Table 3) was conducted using 8 types of antibiotics [29], including aminoglycosides (kanamycin, streptomycin), chloramphenicol, lincosamides (clindamycin), macrolides (azithromycin, erythromycin), quinolones (levofloxacin, gatifloxacin), sulfonamides (sulfamethoxazole/trimethoprim), tetracyclines, and β-lactams (cefpirome, meropenem). According to criteria of the CLSI guidelines [21], strain HN28 was found to be sensitive to cefpirome, chloramphenicol, gatifloxacin, levofloxacin, meropenem, and sulfamethoxazole/trimethoprim, but resistant to the remaining antibiotics. Contrast to standard antibiotic susceptibility testing, WGS can detect novel resistance genes and more accurately determine potential resistance mechanisms, facilitating a precise assessment of multidrug resistance [30]. Therefore, by aligning the HN28 genome with the CARD database, 51 genes resistant to various types of antibiotics, including glycopeptides, lincosamides, macrolides, quinolones, sulfonamides, and tetracyclines, were detected *in silico*. Among them, the most prevalent resistance genes were associated with macrolides,

Table 4
Summary of HN28 genome annotation findings predicted by databases.

Classifications		Results
Genome features		Length (2,280,124 bp)
		Number of genes (2,291)
		GC content (40.83 %)
COG (# of genes involved, percentage of the genome)		Translation, ribosomal structure and biogenesis (199, 11.52 %)
		Carbohydrate transport and metabolism (185, 10.71 %)
		Amino acid transport and metabolism (137, 7.93 %)
		Transcription (131, 7.59 %)
GO	Molecular functions	Catalytic activity (926)
		Binding (802)
	Cellular components	Cellular anatomical entity (567)
	Biological processes	Metabolic process (934)
		Cellular process (891)
KEGG (# of genes involved)		Carbohydrate metabolism (288)
		Membrane transport (151)
		Amino acid metabolism pathways (93)
CaZy (# of genes involved)		Glycoside hydrolases (51)
		Glycosyltransferases (37)
		Carbohydrate-binding modules (12)
PHI (# of genes involved)		Virulence reduction (177)
		No effect on pathogenicity (53)
		Virulence enhancement (17)
		Lethal genes (14)
		Loss of pathogenicity (9)
VFDB	Agglutinin receptor	orf01482
	Plasmin receptor/GAPDH	*plr/gapA* (orf00040)
	Choline-binding protein	*cbpD* (orf00468)
	Fibronectin-binding protein	*pavA* (orf01597)
	Enolase	orf01604
	Trigger factor	*tig/ropA* (orf00768)
	Capsule	orf00911, orf01060-01070, orf01074-01079
	Glutamate dehydrogenase	*gdh*
CARD	(% of the 51 resistant genes involved)	Macrolides (47.05 %)
		Tetracyclines (17.65 %)

constituting 47.05 % of the total (51 resistant genes), with the dominant genotype being *MacB*, followed by tetracyclines, accounting for 17.65 %, primarily including *tetA (46/48/60)* and *tetB (46/60)* genes. The primary mechanism of action involves the active efflux of drugs, effectively eliminating drugs from within the cells [31]. Additionally, strain HN28 also carries four multidrug resistance genes. Comparing the resistance profile with resistance gene analysis, it was observed that the quinolone and sulfonamide resistance genes carried by HN28 do not match the resistance phenotypes.

3.6. Phylogenetic relationship

The genomes of 13 *S. suis* strains were retrieved from the NCBI database, and the phylogenetic tree (Fig. 7) constructed on the basis of SNPs revealed that strain HN28 was closest to NSUI012 (accession number: GCA_034253255.1), a US *S. suis* strain isolated from a septicemic pig. Notably, HN28 shows a distinct phylogenetic relationship from the prevalent domestic ST1 and ST7 strains, belonging to the same lineage as all the other eleven ST25 strains.

4. Discussion

S. suis is an emerging zoonotic agent mainly causing meningitis and septicemia, and a variety of virulence factors has been proposed to be involved in its pathogenesis. *cps* antisera are valuable for distinguishing antigenic differences between strains. Serotyping serves as a crucial epidemiological tool for monitoring *S. suis*, aiding in understanding the diversity and evolution of *cps* within these populations. All *cps* serotypes are believed to be synthesized through the *Wzx/Wzy* pathway [26], and the serotype-specific *wzy* gene has emerged as a promising method for identifying serotypes of *S. suis*. Among all the *S. suis* serotypes derived from human patients, serotype 2 is predominant but phenotypically and genotypically heterogeneous, with ST1 and ST7 as the major ST type when classified by MLST [32,33]. Previous work modeled in animals has shown that ST1 strains are significantly more virulent than ST25 strains.

In this study, a *S. suis* strain of serotype 2 and ST25 (HN28) was isolated from the blood of a septicemic patient, representing the first Chinese septicemic case associated with this type of *S. suis* strain (SS2/ST25). Although strain HN28 is *ef - sly- mrp*$^-$, lacking these three typical virulence genes, pure colonies on the plate from blood culture and recovery after successful antibiotic treatment highlight its close association with this septicemic case, suggesting factors other than the triplet (*ef*, *sly* and *mrp*) might be virulent and pathogenic. As a series of literature commented that *S. suis* is a over-prepared pathogen with multifactorial (and sometimes redundant) virulence factors, some likely performing the same or very similar roles and appearing to compensate for the loss of the other [34–38]. Although the causal relationship of HN28 remains to be determined, its close association with this case is intriguing. One can only speculate rather than be certain how this bacterium caused septicemia in this case without further available experimental data. Since the patient in this study had a history of contacting live pigs/raw pork, HN28 might breach the skin/mucosal barriers of the host, invade through the hematogenous pathway, disseminate in the blood, cause typical septicemic symptoms and eventually lead to hospitalization.

As highlighted in Table 4, annotation results from using various databases indicate that the genome of HN28 strain is predicted to be primarily associated with translation, ribosome structure and biogenesis, and carbohydrate transport and metabolism. Its key molecular functions include catalysis and binding, while the genome is hypothetically engaged predominantly in metabolic and cellular processes. Specifically, it features significant involvement in carbohydrate metabolism, membrane transport, and amino acid metabolism, along with an abundance of carbohydrate enzymes. The genome contains numerous genes associated with low virulence as well as some hypervirulence. Among the putative virulence factors annotated from the genome of strain HN28 (Table 4), four of them were found to be involved in pathogenesis or/and virulence of *S. suis* infections. Specifically, capsule was previously shown to play an essential role in the pathogenesis of most serotypes (including serotype 2) of *S. suis* infections [34–38]; mutation of the glutamate dehydrogenase gene (*gdh*) or trigger factor gene was found to attenuate the virulence of *S. suis* serotype 2 strains [39,40]; enolase could be involved in the pathogenicity of *S. suis* by binding to host cell plasminogen [41]. Although unknown yet in *S. suis*, PavA had a direct role in the pathogenesis of pneumococcal infections [42]. Collectively, these annotations suggest HN28's capability to utilize diverse sugars and to adapt to challenging environments, and reveal the presence of factors potentially crucial for bacterial adhesion, tissue colonization, invasion, and immune evasion. Examination of resistance genes indicates a primary mechanism involving active drug exocytosis, effectively removing drugs from within the cell. These findings collectively support the survival, proliferation, and pathogenicity of *S. suis* within its host, offering valuable insights into its pathogenic mechanisms.

The first and only reported human case associated with an ST25 *S. suis* strain in mainland China was a meningitis patient who consumed raw pork and the bacterium was *ef - sly- mrp* + [43], similar but still distinct from the *ef -sly- mrp*$^-$ profile presented by all the 36 ST25 North American *S. suis* strains in a previous study [34], and the one in the current work.

5. Conclusions

In summary, strain HN28 was isolated from the blood of a septicemic patient and identified as *S. suis* SS2/ST25. On the phylogenetic tree, strain HN28 was closest to strain NSUI012, a USA swine septicemic isolate. Currently, *S. suis* is considered a reservoir for the spread of drug-resistant genes among major *streptococcus* pathogens [44]. Therefore, it is crucial to pay close attention to infections caused by *S. suis* either in clinical research or monitoring of zoonotic pathogens affecting both humans and animals.

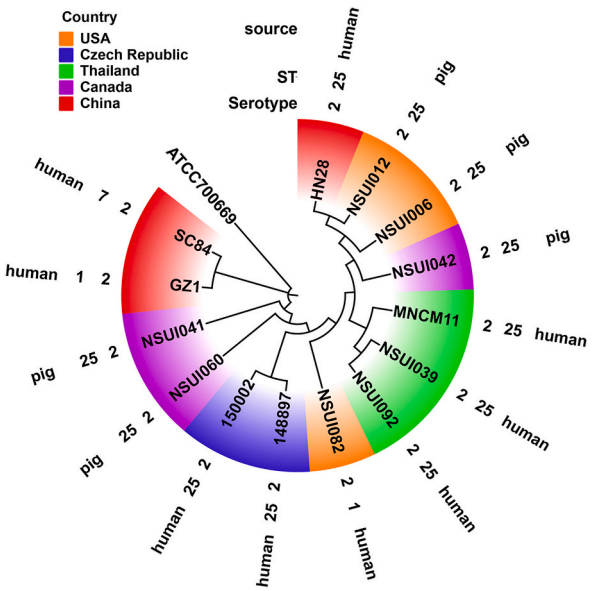

Fig. 7. A maximum-likelihood phylogenetic tree of ST25 population strains based on mutational SNP differences across the 15 genomes and using *Streptococcus pneumoniae* (ATCC 700669) as an outgroup to root the tree. The source, ST, serotype and country origin (color-coded) of the strains are labeled on the outside. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Ethics approval and consent to participate

The study (ethical number HYLL-2022-419, November 24, 2022) was approved by the Second Affiliated Hospital of Hainan Medical University.

Funding statement

This study was supported by the Hainan Province Science and Technology Special Fund (ZDYF2022SHFZ114), National Natural Science Foundation of China (82060377), Hainan Provincial Natural Science Foundation of China (820RC650), the Innovative Research Projects for Postgraduate Students at Hainan Medical University (HYYS2021A22 and Qhys2022-281).

Data availability statement

The datasets supporting our findings are presented in the article.

Verbal informed consent

Because the focus of this study was on strain typing, pathogenicity, and gene function analysis, patient information was used as secondary data, and consent and permission were obtained from the patients for its use and for the disclosure of important patient information and privacy in the article.

CRediT authorship contribution statement

Peipei Cao: Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Meixing Lin:** Data curation, Conceptualization. **Zhiling Chen:** Formal analysis, Data curation. **Guannan Zhang:** Visualization, Data curation. **Xin-He Lai:** Writing – review & editing, Data curation. **Xiang Wu:** Writing – review & editing, Validation, Supervision. **Lina Niu:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35456.

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