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Case Report

Metagenomic next-generation sequencing confirmed a case of sporadic human infection with *Streptococcus suis* in an urban area

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Abstract

Introduction: *Streptococcus suis* (*S. suis*) disease is a zoonotic infection caused by invasive *S. suis* and can lead to meningitis, septic shock, arthritis, and endocarditis. Early treatment is the key to reducing mortality. However, clinical manifestations of most cases are atypical, severely limiting rapid diagnosis and treatment.

Case report: Here, we report a 74-year-old female patient diagnosed with *S. suis* infection. The main symptoms were hearing loss, lumbago, and scattered ecchymosis of the lower extremities and trunk. Blood non-specific infection indexes were significantly increased and platelets were significantly decreased; however, no pathogens were obtained from routine blood culture. Finally, the *S. suis* infection was confirmed by metagenomic next-generation sequencing (mNGS) of blood and cerebrospinal fluid. After antibiotic treatment, the limb and trunk scattered ecchymosis and lumbago symptoms were significantly relieved, but the hearing did not recover.

Conclusions: Human infection with *S. suis* is rare in central cities, and it is easy to misdiagnose, especially in cases with atypical early symptoms. mNGS technology, combined with clinical observation, is helpful to clarify the direction of diagnosis and treatment, which is conducive to patient recovery.

Key words: *Streptococcus suis*; zoonosis; meningitis; metagenome; mNGS.

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Introduction

Streptococcus suis (*S. suis*) is a Gram-positive facultative anaerobic coccus and an important zoonotic pathogen, that usually colonizes the nasal cavity, tonsil, upper respiratory tract, genitalia, and gastrointestinal tract of pigs. In 1968, Danish scholars reported for the first time a case of meningitis caused by *S. suis* in humans [1]. Risk factors for human *S. suis* infection include direct contact with sick or dead pigs, especially those with skin or mucous membrane damage; also, people with occupational contact with pigs or pork are considered susceptible groups. The incubation period of human infection with *S. suis* is 2 to 7 days, and there are no specific clinical symptoms in the early stages of infection. The onset of the disease is rapid, and clinical symptoms and signs are different in severity and presentation. Meningitis and septicemia are the most common clinical manifestations, and other reported clinical manifestations include arthritis, endocarditis, endophthalmitis, and pneumonia [2-4]. Hearing loss is the most common sequelae of *S. suis* infection, and the reported sequelae of hearing loss is 39.1%. In China, the mortality rate among humans infected with *S. suis* is as high as 18% [5]. Considering the severity of the *S. suis* disease, it is necessary to develop rapid and accurate detection methods for *S. suis* to facilitate timely and effective treatments.

Currently, traditional culture methods and serological tests are commonly used for laboratory testing of *S. suis*, followed by molecular diagnostic techniques. *S. suis* must be cultured on a blood agar medium, and the growth cycle is generally complete in 24-48 hours. The culture method is time-consuming, labor-intensive and has low detection sensitivity. *S. suis* shows α-hemolysis on sheep blood plates, and can easily be confused with other common streptococci, such as *Streptococcus viridis*, *Streptococcus bovis*, and *Streptococcus pneumonia* [6-8]. Enzyme-linked immunosorbent assay (ELISA) has the advantages of simple operation, good stability, high specificity, and

speed. However, compared to molecular diagnostic techniques, ELISA has a relatively low sensitivity and is prone to detecting false positives [9]. Polymerase chain reaction (PCR) is the most commonly used molecular diagnostic method, which has high detection sensitivity and requires less time. However, it is more complex to operate than serological tests. Additionally, the PCR detection rate of *S. suis* is significantly higher than that of microscopic examination (41.8% vs. 26.6%) [10].

Metagenomic next-generation sequencing (mNGS) is another molecular diagnostic method that has been increasingly applied in the detection of pathogens, especially in the diagnosis of mixed infections and rare and new pathogens, due to its unbiasedness, high sensitivity, high specificity, and speed [11,12]. Compared to the culture method, pathogen identification with mNGS has a shorter detection cycle (≤ 24 h) and can eliminate the need for clinical assumption of pathogens compared to serum detection and PCR methods. The extensive pathogen detection spectrum of mNGS is more suitable for complex infectious diseases with atypical symptoms; indeed mNGS helps quickly identify pathogens and reduce the screening time. Several publications report the use of mNGS to identify *S. suis* infections in cerebrospinal fluid (CSF) and blood samples [2,13-16]. Here, we report a rare case of meninges infection and thoracolumbar body with local peripheral soft tissue infection caused by *S. sui*s that was detected by mNGS.

Methodology

DNA extraction, library preparation, and metagenomic next-generation sequencing

One mL of plasma and 1 mL of pooled CSF were collected in separate Eppendorf tubes, and total DNA was extracted using the Tiangen Magnetic DNA Kit (Tiangen, Beijing, China) by standard procedures. DNA libraries were constructed using the NEB Next® Ultra™ DNA Library Prep Kit for Illumina® (Illumina, CA, USA). Qubit 2.0 (Invitrogen, CA, USA) was used for quantification, and library quality was subsequently validated with an Agilent 2100 bioanalyzer (Agilent, CA, USA). Finally, the qualified libraries were sequenced using the Illumina NextSeq™ 550 platform (Illumina, CA, USA).

Bioinformatics analysis

Trimmomatic software was used to remove the adapter sequences and low-quality base sequences, and bowtie2 calibration software was used to remove the human host sequences [17,18]. The remaining reads were aligned to the reference database built from multiple public sequence resources of bacteria, viruses, and fungi. The number of reads was standardized using the length of the species genome to calculate their reads per kilobase (RPK). We further calculated the relative abundance of species based on RPK.

Case presentation

A 74-year-old female patient presented with persistent low back pain, weakness in her lower extremities, and unstable standing without apparent cause, three days before admission. The patient lived in an urban area of Shanghai, China, and was not engaged in pig breeding, butchering, or pork retailing but had licked raw pork in the pre-onset. The patient had a history of hypertension, vitiligo, and chronic lumbar degenerative disease. Two days before admission, she was admitted to the emergency department of orthopedics. A lumbar computed tomography (CT) scan showed lumbar disc herniation with lumbar vertebra 3 (L3) spondylolisthesis (degree I), and the patient was then treated with oral analgesics. Her symptoms did not improve during medication administration, and there was nausea and vomiting after eating, accompanied by significant progressive loss of hearing, dizziness, persistent low back pain, significant restriction of waist movement, and no other symptoms such as headache, earache, or fever.

The patient was admitted to our emergency internal medicine department, and the vital signs were stable. On physical examination, there were multiple skin pigmentation losses, ecchymosis in both knees and waist (Figure 1), significant hearing loss, and inability to communicate with speech. There was normal physiological curvature of the spine, and L3-S1 percussion pain was evident. Muscle strength tests

Figure 1. Scattered ecchymosis on the patient's body. (A) Ecchymosis on the patient's knee. (B) Ecchymosis on the patient's waist.

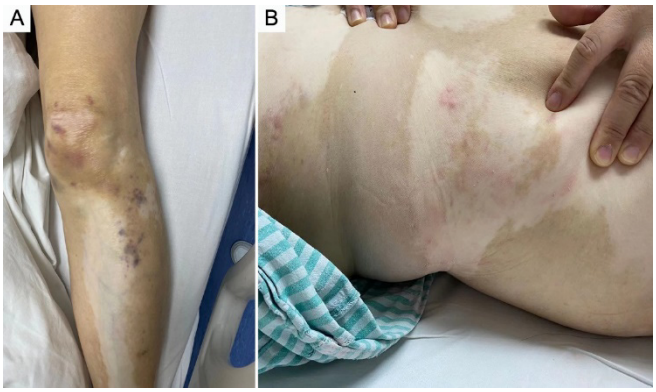


Table 1. Results of laboratory tests performed at the time of admission and on day 44.

Date	Blood routine						Biochemical tests		
	WBC ($\times 10^9$/L)	PLT ($\times 10^9$/L)	N (%)	IL (Pg/mL)	PCT (ng/mL)	CRP (mg/L)	ALT (μ/L)	AST (μ/L)	Cr (μmol/L)
On admission	7.9	50	92	6181.60	34.50	>200.00	49	90	193
Day 44	5.3	677	-	619.51	0.04	27.86	12	18	56

WBC: white blood cells; PLT: procalcitonin; N: neutrophil; IL: interleukin; PCT: procalcitonin; CRP: C reactive protein; ALT: alanine aminotransferase; ASP: aspartate aminotransferase; Cr: creatinine.

showed that the upper limbs were grade V and the lower limbs were grade III, indicating that the muscle strength of both lower limbs was reduced to a lower than standard value. The patient's neck was stiff, had a suspicious positive Kerning's sign, and could not cooperate in completing Brudzinski's sign. The neurologic examination results were negative, which ruled out a neurologic injury. Routine blood examination revealed high inflammatory indicators: total platelet counts of 50×10^9/L, white cell count (WBC) count of 7.9×10^9/L, neutrophil ratio of 92%, C reactive protein (CRP) higher than 200.00 mg/L, and procalcitonin (PCT) count of 34.50 ng/mL. Emergency biochemical examination showed significantly increased levels of transaminase and creatinine; alanine transaminase was 49 μ/L, aspartate transaminase was 90 μ/L, and creatinine was 193 μmol/L (Table 1). Considering the high possibility of infectious diseases, a lumbar infection could not be ruled out, and the patient was empirically treated with antibacterial drug meropenem (0.5 g every 4 h).

Blood samples were sent for mNGS examination on the day after admission. *S. suis* was detected (Table 2) with a genome coverage of 10% on the next day (Figure 2). Linezolid was administered for the following three days to treat the Gram-positive cocci. Chest CT, abdominal CT, and cardiac ultrasound showed no apparent abnormalities. A general examination and mNGS of CSF were performed five days after admission. The mNGS results of CSF also showed presence of *S. suis* (Table 2). Pandy's test of the CSF was positive (1+), and the CSF culture was negative. Meropenem and linezolid were stopped immediately, ceftriaxone (2.0 g every 24 h) with vancomycin (1.0 g every 12 h) were used as therapy. Subsequently, ceftriaxone alone was used. The total course of antibiotics was five weeks. During the disease, dexamethasone (5-10 mg every 24 h) was used intravenously to improve the patient's hearing. Blood cultures were negative on day 1, 7, and 19 after admission. According to the mNGS results of the patient's CSF, the primary infection site was finally considered to be the meninges, and the patient was diagnosed with meningitis.

Two days after admission, lumbar spine and thoracic vertebrae magnetic resonance imaging (MRI) scans revealed herniated discs in chest 2-3, chest 9-10, chest 10-11, and chest 11-12; degenerative changes in thoracic vertebrae; lumbar 3-4, lumbar 4-5 disc herniation, lumbar degenerative changed-anterior spondylolisthesis of lumbar 3 (degree I), and superficial lumbodorsal fasciitis. Lumbar spine and thoracic vertebrae MRI scans were reviewed at 25 days after admission, and the images showed that the patient was still progressing slowly compared with seven days after admission, which we considered an inflammatory disease in the thoracolumbar body with local peripheral soft tissue infection (Figure 3).

After 44 days of hospitalization, the patient's lumbago was significantly relieved, spinal motion improved, skin ecchymosis subsided, body temperature was normal, platelet levels returned to the normal range, inflammatory indexes returned to normal, and liver and kidney function were not significantly impaired (Table 1). Later, the patient was transferred to a superior hospital for diagnosis and treatment. Unfortunately, the patient was discharged with no hearing recovery, and permanent hearing loss was observed during follow-up one year after discharge.

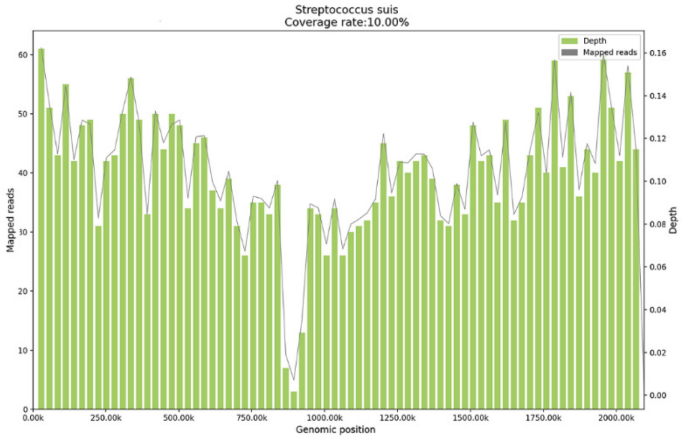
Discussion and conclusions

S. suis can be divided into 35 serotypes based on the different capsular antigens. Some of these serotypes are pathogenic. Serotype 2 has higher transmission and

Table 2. Summary of mNGS results.

Days after admission	Test sample	Genus	Sequence reads (genus)	Species	Sequence reads (species)	Relative abundance
2	Peripheral blood	*Streptococcus*	2534	*Streptococcus suis*	2114	100%
5	Cerebrospinal fluid	*Streptococcus*	1199	*Streptococcus suis*	1144	100%

Figure 2. Genome coverage map of *Streptococcus suis* as detected by mNGS.

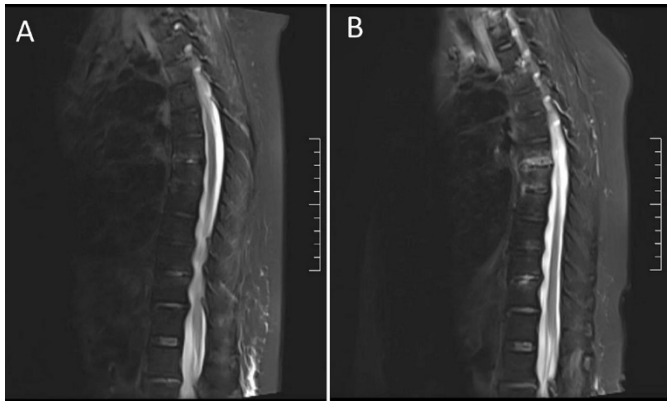


virulence than other serotypes and is the most common serotype that is responsible for human infection [19]. The classification of pathogenic bacteria helps to prevent and control the spread of epidemics caused by these bacteria. Multilocus-sequence typing (MLST) is commonly used to determine the serotype of pathogenic bacteria, including *S. suis* [20]. With the reduction of genome sequencing costs and the advancement of bioinformatics technology, whole genome multi-locus sequence typing (wgMLST) based on whole genome sequencing is becoming the *de facto* standard for bacterial typing [21,22]. As an emerging technology, mNGS has demonstrated many advantages for epidemiological studies and antibiotic resistance prediction; but there are still some challenges. Currently, mNGS is rarely used for strain-level genotyping in clinical practice because of its random fragment sequencing and low coverage, which may lead to the omission of essential housekeeping genes and thus affect the typing accuracy. Further improvements in genome coverage with capture probe technology or the use of specific typing tools suitable for low sequencing depths will make it possible to type the detected pathogens [23,24]. The low coverage of mNGS may also make it possible to detect drug-resistance and virulence genes [25]. In addition, the short reads of next-generation sequencing make it difficult to determine which pathogen a detected resistance gene belongs to and whether it is located on a mobile genetic element.

In China, *S. suis* has so far been identified as the causal agent in sporadic cases of human infection. *S. suis* has been reported in Jiangsu, Shandong, and Hebei provinces in the past two years. As a megacity with a largely disappearing pig farming industry, only two cases of local infection with *S. suis* have been reported in Shanghai, in 2014 and 2018 [26]. In the current case, the patient lived in urban areas and was not at high risk of *S. suis* infection like pig farmers and meat processors. The patient's first symptom was lower back pain. The patient had a history of chronic lumbar degenerative disease, and the early infection symptoms were not obvious. Therefore, the patient first went to the orthopedics department without considering that it might be an infectious disease. It was difficult to diagnose whether it was an infection based only on the imaging results, which delayed the diagnosis. In 2022, a spinal canal infection caused by *S. suis* was reported, suggesting that patients with fever, headache, and lower back pain may also have *S. suis* [15]. In this case, the patient had a history of chronic degeneration of the lumbar spine, which might also be one of the causes of lower back pain. Interestingly, the patient had the symptoms of subcutaneous ecchymosis, which is one of the early symptoms of human infection with *S. suis*, although it has rarely been reported [14].

The patient's symptoms did not improve after taking conventional analgesics, and the disease progressed rapidly, with toxic symptoms such as skin ecchymosis, nausea, vomiting, and apparent hearing loss. Given the rare zoonotic infections in urban Shanghai and the lack of understanding of the disease species, it was difficult to explain the clinical manifestations of the patient during the initial treatment. No pathogen was detected by conventional laboratory tests during the entire process of diagnosis. Multiple blood cultures and cerebrospinal fluid cultures were negative, which might be related to the sensitivity of *S. suis* to most antibiotics used before culture [14]. We were able to identify *S. suis* in the CSF and blood, and show that meningitis and bacteremia in patients were the result of *S. suis* infection using the mNGS technology. Meningitis is the most

Figure 3. Thoracic vertebrae imaging. (A) MRI of thoracic vertebrae taken 7 days after admission. (B) MRI of thoracic vertebrae taken 25 days after admission.



common clinical manifestation of *S. suis* infection, and the symptoms are similar to those of other types of bacterial purulent meningitis. The accurate diagnosis with mNGS in the shortest possible time allowed us to identify the infectious pathogens and manage theclinical antimicrobial drug use. Unfortunately, the patient's spine showed slow progression and imaging findings lagged behind clinical practice during hospitalization. It was unclear whether the infection had been controlled or whether there were other causes of infection.

It is difficult to effectively identify, using conventional methods, the pathogen in patients with infectious diseases, especially when the patients have used broad-spectrum antibiotics in the early stages of the disease. mNGS testing can be considered in such cases. Human infection with *S. suis* is rare in central cities, especially cases with atypical symptoms which are easy to misdiagnose. The mNGS technology can be used to identify the pathogen and this information can be used to establish the direction of diagnosis and the best treatment approach, leading to the recovery of the patients.

Ethics statement

Written informed consent was obtained from the patient.

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