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The value of next-generation sequencing for the diagnosis of *Streptococcus suis* meningitis

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SUMMARY

OBJECTIVE: The aim of this study was to investigate the value of next-generation sequencing for the diagnosis of *Streptococcus suis* meningitis.

METHODS: Patients with meningitis in the Department of Neurology of the Hainan General Hospital were recruited and divided into a next-generation sequencing group and a control group. In the next-generation sequencing group, we used the next-generation sequencing method to detect the specific pathogenic bacteria in the patients. In the control group, we used the cerebrospinal fluid bacterial culture method to detect the specific pathogenic bacteria in the patients.

RESULTS: A total of 28 participants were recruited for this study, with 14 participants in each group. The results showed similarities in both the average age and average course of the disease between the two groups ($p>0.05$). The white blood cell count, percentage of neutrophils, and level of C-reactive protein in the next-generation sequencing group were significantly higher than those in the control group ($p<0.05$). There were similarities in both the temperature and intracranial pressure between the two groups ($p>0.05$). In the next-generation sequencing group, all patients (100%) were detected as having had the *S. suis* meningitis infection by next-generation sequencing, while only 6 (43%) patients in the control group had been detected as having the *S. suis* meningitis infection by cerebrospinal fluid bacterial culture.

CONCLUSIONS: The positive detection rate of *S. suis* by the next-generation sequencing method was significantly higher compared with using a cerebrospinal fluid bacterial culture. Therefore, the next-generation sequencing method is valuable for the diagnosis of *S. suis* meningitis and is worthy of clinical application.

KEYWORDS: Streptococcus suis. Cerebrospinal fluid. Next-generation sequencing. Meningitis. Bacterial culture.

INTRODUCTION

Streptococcus suis (*Ss*) meningitis is an acute infectious disease caused by *Ss,* invading the central nervous system. The main form of transmission is through close contact between people and pigs; the pathogen invades the body through damaged skin or mucous membranes and infects the central nervous system via the blood. It is the primary type of *Ss* infection among humans, and its main clinical symptoms include fever, headache, and hearing loss, and in severe cases, consciousness disturbances may occur[1]. Most of the cerebrospinal fluid of patients with *Ss* meningitis showed purulent meningitis[2], and some patients reflected similar viral meningitis[3] or tuberculous meningitis[4].

Next-generation sequencing (NGS) is a large-scale parallel sequencing technology that allows for thousands to billions of deoxyribonucleic acid (DNA) fragments to be simultaneously and independently sequenced[5]. Since most infectious agents include DNA or RNA genomes, existing research findings on NGS technology have been widely applied to infectious diseases. Studies have shown that the application of NGS to infectious disease outbreaks of unknown causes and patients with suspected infections has positive results[6]. The applications of NGS included whole-genome sequencing of microbial isolates, microbiome studies, drug resistance testing of viruses or culture isolates, and lineage tracing[7]. Additionally, NGS technology exhibited efficient potential through unbiased pathogen detection. It has been demonstrated in the field of microbiology and in clinical contexts, and its application is rapidly becoming routine[8].

Traditional blood culture and cerebrospinal fluid culture detection of pathogens have the disadvantages of long detection times and low positive rates. This can make it easy to misdiagnose and affect patients' prognoses. Cerebrospinal fluid pathogen NGS technology is an emerging molecular diagnostic method that can quickly detect intracranial pathogens. The literature

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evidenced its use in the diagnosis of encephalitis; however, its applicability for routine diagnosis has not been evaluated[9]. Accordingly, this study investigated the value of NGS for the diagnosis of *Ss* meningitis.

METHODS

Participants

From August 2018 to January 2021, patients with meningitis in the Department of Neurology of the Hainan General Hospital were recruited and divided into an NGS group and a control group. This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of our hospital. All participants signed informed consent forms for inclusion in the study.

The diagnostic criteria of *Ss* meningitis are as follows:

1. Coma may occur in patients with acute onset, fever, chills, general discomfort, fatigue, headache, dizziness, nausea, and vomiting (possibly jet vomiting). There are no bleeding spots, ecchymosis spots, and ecchymosis spots on the skin. No shock.
2. The meningeal stimulation sign was positive, and the cerebrospinal fluid showed suppurative changes.
3. The NGS or cerebrospinal fluid bacterial culture method was used to detect specific pathogens in patients.

Inclusion and exclusion criteria

The inclusion criteria were as follows:

1. Patients who had been diagnosed with meningitis;
2. Those older than 18 years;
3. Patients suspected of suffering from *Ss* meningitis:
   a. recent history of close contact with sick pigs and their products, especially those with damaged skin and mucosa;
   b. acute onset, chills, fever, and other acute infectious poisoning symptoms; and
4. Patients who provided signed informed consent for inclusion in the study.

The exclusion criteria were as follows:

1. Patients who had an advanced malignant tumor and
2. Patients whose data were incomplete.

METHODS

In the NGS group, we used the NGS method to detect specific pathogenic bacteria in the patients. In the control group, we used the cerebrospinal fluid bacterial culture method to detect the specific pathogenic bacteria in the patients.

Cerebrospinal fluid detection

For the lumbar puncture, approximately 2–3 mL of cerebrospinal fluid was collected, sterilely sealed, and stored at -20°C or transported on dry ice to Yuguo Biotechnology (Beijing) Co., Ltd., for metagenomics NGS detection. Following the TIANGEN DNA Mini kit DP316 (TIANGEN Biotechnology Company, Beijing, TIANGEN DNA) instructions, 200 μL of cerebrospinal fluid was collected to extract DNA and purify it. Nucleic acid concentration and quality were detected and confirmed using the Qubit dsDNA HS kit (Thermo Fisher Scientific Co., Ltd., Shanghai) and agarose gel electrophoresis.

The Qiagen library construction kit (QIAseq™ Ultralow Input Library Kit) operation manual was followed to complete the DNA library construction. The Qubit dsDNA HS kit (Thermo Fisher Scientific Co., Ltd.) and agarose gel electrophoresis were used to detect DNA concentration and quality. Qualified DNA libraries with different barcode tags were collected and sequenced by the Illumina NextSeq sequencing platform.

After obtaining the sequencing data, high-quality data were generated by filtering out joints, low-quality, low-complexity, and short sequences. Then, the human sequence that matched the human reference genome was removed using the SNAP software. Burrow-Wheeler alignment was used to compare the remaining data with the microbial genome database. The database collects a large number of microbial genomes from NCBI, including more than 20,000 microorganisms, 11,910 bacteria species, 7,103 virus species, 1,046 fungi species, and 305 parasite species. Finally, the microbiological composition of the sample is obtained. It took approximately 2–3 days to obtain the NGS microbial identification results from the cerebrospinal fluid.

Main observation indexes

In this study, the main observation indexes included sex, age, the course of the disease, temperature, white blood cell count, the percentage of neutrophils, the level of C-reactive protein, intracranial pressure, and cerebrospinal fluid detection.

Statistical analysis

This study used the SPSS Statistics version 20.0 (IBM, Chicago, IL, USA) software to conduct the statistical analysis. The continuous variables of normal distribution were expressed as mean±standard deviation, the continuous variables of non-normal distribution were expressed as a median

(interquartile range), and the categorical variables were expressed as a frequency (percentage [%]). For the two comparisons, each value was compared using a t-test when each datum conformed to a normal distribution, while non-normally distributed continuous data were compared using nonparametric tests. The counting data were tested by the chi-square test; $p<0.05$ was considered statistically significant.

RESULTS

General characteristics

A total of 28 participants were recruited for this study, including 4 females and 24 males. There were 14 patients in the NGS group; the average age was 54.79±10.34 years and the average course of the disease was 5.93±3.52 days. There were 14 patients in the control group; the average age was 46.71±18.96 years and the average course of the disease was 8.07±7.49 days. The results showed similarities in both the average age (p=0.151) and the average course of the disease (p=0.350) between the two groups.

Comparison of routine and biochemical indexes between the two groups

In the NGS group, the temperature was 39.10±0.44°C, the white blood cell count was $12.45±4.21×10^9$, the percentage of neutrophils was 82.48±8.50%, and the level of C-reactive protein was 134.07±88.86 mg/L. In the control group, the temperature was 38.46±0.98°C, the white blood cell count was $7.95±2.91×10^9$, the percentage of neutrophils was 71.99±9.23%, and the level of C-reactive protein was 4.70±6.03 mg/L. The results showed that the white blood cell count (p=0.005), the percentage of neutrophils (p=0.004), and the level of C-reactive protein (p<0.001) in the NGS group were significantly higher than in the control group. There were also similarities in temperature (p=0.082) between the two groups. The details are shown in Figure 1.

Cerebrospinal fluid test results between the two groups

In the NGS group, the intracranial pressure was 214.29±67.14 mmH_2O. In the control group, the intracranial pressure was 190.86±55.99 mmH_2O. The results showed similar intracranial pressure between the two groups (p=0.350).

In the NGS group, all patients (100%) had been detected as having the *Ss* meningitis infection by NGS, while only 6 (43%) patients in the control group had been detected as having the *Ss* meningitis infection by cerebrospinal fluid bacterial culture.

DISCUSSION

S. suis is a Gram-positive and anaerobic zoonotic pathogen that can be divided into 35 serotypes, the most common of which is serotype II. Anyone is susceptible to the *Ss* pathogen and, following infection, it can cause meningitis (the most common manifestation), sepsis, endocarditis, and arthritis. Most of these conditions can lead to serious cochlear nerve and vestibular nerve damage sequelae[10]. The pathogen enters the central nervous system through the brain microvascular epithelial cells or choroidal epithelial cells via the blood-brain barrier or blood-cerebrospinal fluid barrier and causes inflammation of the meninges and brain parenchyma[11]. The main pathogenic

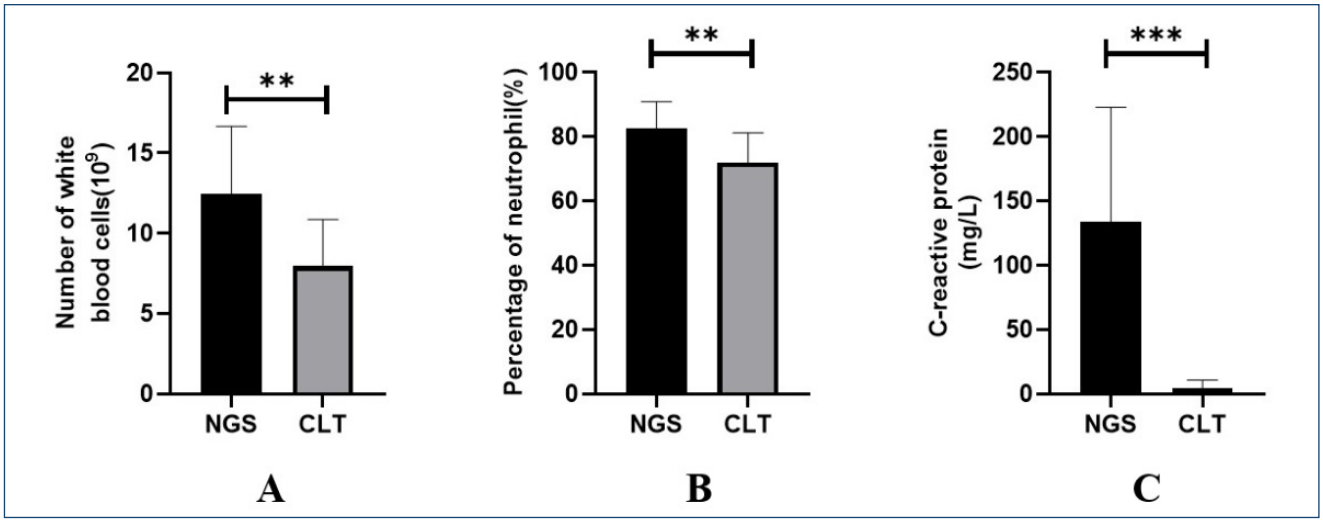

Figure 1. (A) The comparison of white blood cell between the two groups. (B) The comparison of the percentage of neutrophils between the two groups. (C) The comparison of the level of C-reactive protein between the two groups. **p<0.01. ***p<0.001.

risk factors include occupational exposure to pigs and raw pork or eating raw pork products[12]. The diagnosis of this disease depends on the positive culture of *Ss* in the blood-cerebrospinal fluid[13]. However, due to factors such as low blood-cerebrospinal fluid, insufficient sampling, or early antibiotic treatment, the positive rate of blood-cerebrospinal fluid cultures is low, and the positive rate of bacterial blood cultures is approximately 32%. The positive rate of cerebrospinal fluid bacterial cultures is 18.5%, leading to a higher incidence of poor prognosis[14]. Early diagnosis and treatment can help improve the clinical outcome of patients. Therefore, a rapid and accurate detection method is needed to assist with diagnosis in clinical practice. The NGS of cerebrospinal fluid is used as a molecular diagnosis of infectious diseases of unknown pathogens. This new technology has attracted significant attention[5].

The common symptoms and signs of *Ss* meningitis include fever, headache, and neck stiffness[15]. Bilateral hearing loss can occur during early onset with an incidence as high as 66.4%[16]. All five patients had a fever, headache, and bilateral deafness. The incidence was 100%, which was higher than noted in existing literature reports[15,16]. Reports in the literature showed that the classification of cerebrospinal fluid white blood cells in this disease was dominated by multiple nuclear cells, which were transformed into mononuclear cells or lymphocytes quickly after antibiotic treatment[2]. If the epidemiological data of patients are not clear, the condition can be easily confused with tuberculous meningitis, resulting in a misdiagnosis.

The NGS of cerebrospinal fluid pathogens as a molecular technology to assist in the diagnosis of central nervous system infections can quickly and sensitively identify pathogens[17]. This can enable clinicians to make reasonable interpretations of test results, based on a patient's clinical manifestations, and select an appropriate antibiotic treatment plan[18].

The results showed that all patients (100%) had been detected as having the *Ss* meningitis infection by NGS in the NGS group, while only 6 (43%) patients in the control group had been detected as having the *Ss* meningitis infection by cerebrospinal fluid bacterial culture. Therefore, the positive detection rate of *Ss* using the NGS method was significantly higher compared with detection via cerebrospinal fluid bacterial culture.

This study had multiple limitations. First, it was not a randomized controlled trial. Second, the sample size was limited and, as such, a larger trial with more participants is necessary for further study.

CONCLUSIONS

The positive detection rate of *Ss* by the NGS method was significantly higher compared with detection via cerebrospinal fluid bacterial culture. Therefore, the NGS method represents value for the diagnosis of *Ss* meningitis and is worthy of clinical application.

AUTHORS' CONTRIBUTIONS

EZ: Conceptualization, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing. **DW:** Formal analysis, Writing – original draft, Writing – review & editing. **ZZ:** Data curation, Writing – original draft, Writing – review & editing. **LX:** Data curation, Software, Writing – original draft, Writing – review & editing. **XH:** Software, Writing – original draft, Writing – review & editing. **PH:** Validation, Writing – original draft, Writing – review & editing. **FO:** Investigation, Writing – original draft, Writing – review & editing. **GW:** Resources, Writing – original draft, Writing – review & editing. **SH:** Supervision, Writing – original draft, Writing – review & editing. **YG:** Methodology, Writing – original draft, Writing – review & editing.

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