Detecting *Streptococcus suis* by nanopore sequencing in endophthalmitis: A case report

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**Abstract**

Endophthalmitis is a rare and infectious disease caused by *Streptococcus suis* (*S. suis*). Traditionally, *S. suis* is detected by the pathogenic microorganism culture method, which has low positivity and high false negativity. Nanopore sequencing (NS), which is a third-generation sequencing technology, has several advantages over the traditional method; in particular, it is cost and time effective and has a high throughput. In this report, a case of infectious endophthalmitis caused by trauma is examined. The NS results suggest that the pathogen in question is a mixed infection caused by *S. suis* and *Clostridium perfringens*. This case report provides evidence of the fact that NS can quickly identify pathogens, which is of great significance for clinical diagnosis and treatment.

**Keywords**
bacterial culture, case report, endophthalmitis, nanopore sequencing, *Streptococcus suis*

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**Introduction**

*Streptococcus suis* (*S. suis*) is a type of normal microflora that commonly exists in the respiratory and digestive tract of pigs. It is a Gram-positive and facultative Anaerococcus type of bacterium. Some strains of *S. suis* can infect mammals other than pigs, which means that *S. suis* is an important zoonotic pathogen. For humans infected with *S. suis*, the most common symptoms are meningitis and septicemia; endophthalmitis is rare, with 4.6% of people showing symptoms.

At present, the gold standard for diagnosing endophthalmitis is the detection of etiology in the intraocular fluid. Generally, the positivity of the traditional pathogen culture method is low, which is easily influenced by a number of factors, such as culture duration and environment. Nanopore sequencing (NS), which is a third-generation sequencing technology, recognizes the DNA sequence by the current change of a single DNA strand passing through a single nanopore protein on the resistance membrane after said DNA strand is unwound. It is highly efficient with respect to detecting pathogenic microorganisms. This paper reports a case of endophthalmitis caused by *S. suis* that was detected by NS.

**Case data**

A male patient (22 years old) was admitted to emergency on January 16, 2019, complaining about “post-traumatic pain in the right eye with vision loss for 5 h.” At the time of admission, he reported...
that his right eye was stabbed by a pigsty bamboo pole 5 h earlier. The following symptoms were present: redness of the eye and ophthalmodynia as well as blurred and decreased vision. The patient came to our hospital for emergency treatment after simple bandaging in a local hospital. The physical examination revealed that the visual acuity of the right eye was counting the fingers in front of the eye, and the visual acuity of the left eye was 0.2 (corrected visual acuity of 1.0). Near the limbus of the cornea, two corneal lacerations were present in the upper temporal part of the right eye (roughly 5 mm in length), and there were gray incarcerations of foreign bodies and iris tissue. Hematocele and exudative membrane were visible in the anterior chamber, with a slightly turbid lens and unclear details of vitreous body and fundus. Intraocular pressure was not detected in the right eye; finger intraocular pressure was measured in Tn. The plain computed tomography scan of the orbit did not identify fracturing nor foreign bodies in both eyes. The blood routine test revealed a white blood cell count of 13.67 × 10^9/L and a neutrophil percentage of 86.90%. The primary diagnosis was corneal perforating injury with foreign body incarceration and hyphema in the anterior chamber of the right eye. Debridement and suturing of the right cornea was conducted under local anesthesia, and vancomycin (1/0.1 mg·ml⁻¹) and ceftazidime (2/0.1 mg·ml⁻¹) were injected into the vitreous cavity.

During morning rounds the next day, the patient’s right cornea had foggy edema and a large amount of pus in the anterior chamber. Considering the existence of bacterial endophthalmitis in the right eye, “Lensectomy + Vitrectomy of the right eye + Silicone Oil Filling” was immediately conducted under local anesthesia. Vancomycin (1/0.1 mg·ml⁻¹) and ceftazidime (2/0.1 mg·ml⁻¹) were administered again. After removing the pus in the anterior chamber and the turbid lens during the operation, the vitreous cavity was yellow and white turbid; moreover, extensive retinal hemorrhaging was present as well as frost-like changes in the retinal vascular branches. Fluid samples in the aqueous humor and vitreous fluids were collected during the operation for bacterial and fungal culture, drug sensitivity test, and NS. Local and systemic anti-infection therapy was adopted postoperatively. Tobramycin and dexamethasone ophthalmic suspension and ceftazidime eye drops were administered on the operative side four times per day, and tobramycin and dexamethasone ophthalmic ointment and atropine eye gel were applied once every night. Levofloxacin hydrochloride sodium chloride (0.4 g) was injected once per day, and cefmendazole sodium chloride (2 g) was injected once every 12 h for seven consecutive days.

Intraocular fluid samples obtained during surgery were sent to the laboratory for nanopore sequencing according to the method described by Wang et al.³ The 16S rDNA, ITS1/2 (interfungal transcriptional region 1 and 2), and rpoB genes from the same sample were amplified using QIAamp UCP DNA extraction Kit, and the amplified products were fused at a 10:3:1 mass ratio. The mixed products from different samples were then evenly mixed and used to construct sequencing libraries using a 1D connection kit (SQK-LSK109). The library was sequenced with the Oxford Nanopore MinION or GridION platform. TE buffer was used as the negative control for each batch of determination. Reads with low quality (Q value <7), excessively long or excessively short length (length <200 nt or length >2000 nt) were discarded. Remaining reads were pruned using Porechop (v0.2.4). Finally, Using BLAST to map reads of each sample against 16S rDNA/ITS reference database collected from NCBI FTP (ftp://ftp.ncbi.nlm.nih.gov/refseq/TargetedLoci), the reads preliminarily incorporated into the same species, a consensus sequence was generated using Medaka (v. 0.10.1), then the consensus sequence with 16s rDNA/ITS reference database to compare again to get the final test results. After bioinformatics analysis, a bacterial or fungal test indicates a positive result if the sample reaches any given threshold. According to published literature and clinical guidelines, detected microorganisms have been described as key pathogens, opportunistic pathogens, or typical non-pathogenic symbiotic microorganisms.⁴

Twelve hours later, the NS detected S suis and Clostridium perfringens in the aqueous and fluids in the vitreous body. The number of sequences for S suis and Clostridium perfringens were 5566 and 3392, respectively (Supplemental Information 1). The coverage of detection data and reference sequence was more than 95%. Forty-eight hours later, the pathogen culture identified Streptococcus viridis and Bacillus cereus in the aqueous humor and vitreous fluid. Due to the traumatic history of the right eye being stabbed by a bamboo pole in a pigsty, and the NS results, S suis and Clostridium
perfringens were identified as the pathogens responsible for the endophthalmitis.

Two days after surgery, the corneal edema in the right eye became alleviated, but exudation in the anterior chamber was still obvious (Figure 1). The aforementioned anti-infection treatments were continued until the eye infection gradually improved. Eight days after surgery, the physical examination showed that the visual acuity of the injured eye was counting fingers in front of the eye, the cornea was basically transparent, the exudate of the anterior chamber was completely absorbed, the lens was absent, the retina was flat, and the intraocular pressure was normal. Fifteen days after surgery, the injured cornea was transparent, the anterior chamber was clear, the vitreous cavity was filled with silicone oil, and the retina was flat. Silicon oil removal was conducted 3 months later, and the visual acuity of the right eye was corrected to 0.02 with an intraocular pressure of 18 mm Hg and a flat retina (Figure 2). The patient is still undergoing follow-up appointments.

Discussion

In the traumatic endophthalmitis, coagulase-negative Staphylococcus of the Gram-positive cocci is the most common pathogenic bacterium; endophthalmitis caused by S suis is rare. In 1978, McLendon et al.\(^5\) reported the first case of infectious endophthalmitis caused by S suis. In 2019, Rayanakorn et al.\(^6\) reported a case of S suis infection in a 48-year-old Thai male due to eating raw pork products, which resulted in the occurrence of endogenous endophthalmitis. In the present case report, the right eye of the patient was stabbed by a pigsty bamboo pole before admission; the cornea was full-thickness lacerated, and endophthalmitis was evident, caused by S suis.

At present, the diagnosis of infectious endophthalmitis mainly depends on the clinical manifestations and physical signs of patients. The gold standard for diagnosis is the detection of the pathogen in the intraocular fluid. The traditional pathogen microorganism culture method is influenced by a number of factors, such as culture duration and environment as well as the use of antibiotics before the culture. In addition, the number of samples that can be safely obtained from the eye is small, and some of the caustic bacteria are difficult to survive in the culture medium, so the positivity of the culture is often low (approximately 40%–70%).\(^7\) The diagnosis of infectious endophthalmitis cannot always be ruled out, even if the culture results are negative.\(^8\) With an increased understanding of DNA structure, the rapid development of DNA sequencing technology, and the reduction in detection costs, gene sequencing has become a vital detection method in the field of etiological diagnosis. For instance, NS can recognize the DNA sequence due to the current change generated by a single unwound DNA strand passing through a single nanopore protein on the resistance membrane. Compared with second-generation sequencing technology, NS has a longer sequence reading length; moreover, it conducts real-time analysis of the generated data, thereby reducing the time required for the detection process. In addition, we only need a small amount of sample to detect the potential pathogens, which greatly improves the detection rate. NS is increasing in popularity due to its high throughput and cost and time effectiveness.\(^9\)–\(^10\) Compared with the traditional microbial culture method, NS has obvious advantages in the detection of rare and mixed bacterial infection. From the present case report (and previous reports), it is evident that S suis is often reported as Streptococcus viridis when the traditional microbial culture method is used, which, in turn, results in a low detection rate of S suis.\(^11\) For the present case study, the culture results suggested a mixed infection of Bacillus cereus, which is inconsistent with the Clostridium perfringens detected by NST. Both Bacillus cereus and Clostridium perfringens
are Gram-positive bacteria with similar physical characteristics, which makes it difficult to distinguish between the two under a microscope.\textsuperscript{12} NS can recognize the DNA sequence of these two kinds of bacteria, which means that it is more reliable than the traditional microbial culture method.

The “Lensectomy + Vitrectomy of the right eye + Silicone Oil Filling” was immediately conducted for the patient in question, and vancomycin and ceftazidime were again injected into the vitreous cavity. Fifteen days after surgery, the injured cornea was transparent, the anterior chamber was clear, and the retina was flat. Three months after surgery, the visual acuity of the right eye was corrected to 0.02 with an intraocular pressure of 18 mm Hg, and the retina was flat. In terms of treatment, vancomycin, and ceftazidime were injected into the vitreous cavity during the emergency corneal debridement and suturing operation; however, severe infectious endophthalmitis, foggy corneal edema, and a large amount of pus in the anterior chamber were present on the second day, which suggest that the disease had progressed rapidly. This can be attributed to the high virulence caused by the mixed infection of S suis and Clostridium perfringens. Strong exotoxins can rapidly destroy a

\textbf{Figure 2.} The condition of the right eye half a month and half a year after the vitrectomy: (a) half a month after the vitrectomy, the cornea of the injured eye was transparent, the anterior chamber was clear, and the vitreous cavity was filled with silicone oil, (b) and (c) follow-up at half a year after the trauma, the silicone in the injured eye was removed with a flat retina in the fundus.
significant amount of eye tissue, and, accordingly, patients often have poor prognosis. Early administration of vitrectomy can save patient vision (to a certain extent) and preserve the eyeball.13

**Conclusion**

Our results suggest that the pathogen in question is a mixed infection caused by S suis and Clostridium perfringens. This case report provides evidence of the fact that NS can quickly identify pathogens, which is of great significance for clinical diagnosis and treatment.

**Declaration of conflicting interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Ethical approval**

Ethical approval for this study was obtained from *ETHICS COMMITTEE of Union Hospital Affiliated with Tongji Medical College of Huazhong University of Science and Technology.*

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**Informed consent**

Written informed consent was obtained from all subjects before the study.

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**Supplemental material**

Supplemental material for this article is available online.

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