• Review Article •

Nanopore techniques as a potent tool in the diagnosis and treatment of endophthalmitis: a literature review

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Abstract

● Endophthalmitis is a serious ophthalmic disease characterized by changes in the eye's posterior segment, such as hypopyon and intraocular inflammation, vitritis being a hallmark. Infection-caused endophthalmitis can lead to irreversible vision loss, accompanied by eye pain or eye distention, and in the most severe cases the removal of the eyeball. Microorganisms such as bacteria, fungi, viruses, and parasites typically account for the disease and the entry pathways of the microbial can be divided into either endogenous or exogenous approaches, according to the origin of the etiological agents. Exogenous endophthalmitis can be derived from various occasions (such as post-operative complications or trauma) while endogenous endophthalmitis results from the bloodstream which carries pathogens to the eye. This review aims to summarize the application of new technology in pathogen identification of endophthalmitis so as to prevent the disease and better guide clinical diagnosis and treatment.

● **KEYWORDS:** postoperative endophthalmitis; high-throughput sequencing; metagenomics; long-read nanopore targeted sequencing; pathogen identification

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INTRODUCTION

Endophthalmitis is a serious ophthalmic disease characterized by some changes in the posterior segment of the eye such as hypopyon and intraocular inflammation, vitritis being a hallmark[1]. Infection-caused endophthalmitis can lead to irreversible vision loss, accompanied by eye pain or eye distention, and in the most severe cases the removal of the eyeball, in which process the hosts' response to infection plays an important role in causing endophthalmitis-related damage[2]. Microorganisms such as bacteria, fungi, viruses, and parasites typically account for the disease and the entry pathways of the microbial can be divided into either endogenous or exogenous approaches, according to the origin of the etiological agents. Exogenous endophthalmitis can be derived from various occasions (such as post-operative complications or trauma) while endogenous endophthalmitis results from the bloodstream which carries pathogens to the eye[3].

High-throughput sequencing (HTS), a gene detection means with a high success rate and low costs, shows great utility in the clinical analysis of ocular samples and detection of pathogens in patients diagnosed with endophthalmitis[4]. HTS technology is also called the next-generation sequencing (NGS) technologies, which are specifically divided into second and third-generation sequencing technologies[5]. As an application for HTS, metagenomic NGs (mNGS) is a powerful technology that can simultaneously achieve the qualitative and quantitative identification of pathogens in endophthalmitis, which is more accurate and has revealed wide prospects for identifying pathogens that have not been previously identified, compared with traditional pathogen culture[6-7]. There has been much discussion of the advantages and disadvantages of metagenomic techniques, the lack of corresponding reference gene sequence databases, technical difficulties of metagenome assembly, and phasing in heterogeneous environmental samples being some of its disadvantages. The advantages that are most conducive to clinical occasions include greater resolution of species and strains across phyla and functional content[8-9].

In recent years, the nanopore sequencing technology, known as the fourth-generation sequencing technology, has become a major breakthrough in the realm of genome sequencing after the second and third-generation sequencing technology. Owing to the emergence of the long-read nanopore sequencer MinION developed by Oxford Nanopore Technologies (ONT) and later the sequencer PromethION with improved throughput, rapid pathogens detection can be achieved, despite

its high error rate at the base level, the need of repetitive tests, optimized signal noise ratio (SNR), more reader heads and so forth to be improved[10-12]. Besides, the application of long-read technology has made it possible to interpret complex genomes and obtain metagenomic assembled genomes, especially conducive to the study of microbial communities[13].

This review aims to summarize the application of various molecular techniques in the diagnosis and treatment of endophthalmitis.

MATERIALS AND METHODS

In order to describe in detail the importance of new technologies in the diagnosis, treatment and prognosis of endophthalmitis, we searched the literature through PubMed and Web of Science databases extensively from 1998 to 2022 by using the keywords (infectious endophthalmitis, culture, metagenomics, nanopore sequencing). The following query conditions were applied: 1) (infectious endophthalmitis) AND (culture); 2) (infectious endophthalmitis) AND (metagenomics); 3) (infectious endophthalmitis) AND (nanopore sequencing). Most relevant results were manually screened and categorized by Li ZY. In this literature review, we summarized the microbial composition of endophthalmitis, and compared the traditional culture method with the molecular diagnostic technology, showing the superiority of molecular sequencing technology in detecting microorganisms.

RESULTS

Diagnosis of Endophthalmitis

Microbiological composition of endophthalmitis Exogenous endophthalmitis is the most common, while eye surgery is a major cause of endophthalmitis. Postoperative endophthalmitis can be divided into non-infectious (sterile) endophthalmitis and infectious endophthalmitis. Infectious endophthalmitis is mainly caused by microorganisms while non-infectious endophthalmitis can be attributed to several causes, including postoperative retained soft lens material or drug toxicity in the eye[3]. According to literature reports, among all the surgical causes of exogenous endophthalmitis, cataract surgery accounted for the largest proportion, followed by lens implantation, vitrectomy, penetrating keratoplasty, glaucoma drainage device, trabeculectomy, and intravitreal injection[14]. However, a series of studies have shown a downward trend in the incidence of endophthalmitis after cataract surgery[15]. Acute-onset endophthalmitis occurred in 0.04% of 8 542 838 cataract surgeries performed in the United States between 2013 and 2017[16]. In addition, some external eye surgeries can also cause endophthalmitis[17].

The widespread use of eye surgery has also led to a series of changes in the overall microbiome of the eye[18]. Ong *et al*[19] analyzed changes in the bacterial community patterns of endophthalmitis after cataract surgery or intravitreal injection, revealing *Staphylococcus epidermidis* as the most common pathogen. This supports some previous experimental findings[20-21]. Dave *et al*[22] investigated the cases of infectious endophthalmitis after evisceration of the eyeball. Vitreous samples were obtained and their microbial spectrum and antibiotic susceptibility were analyzed. They found that gram-positive bacteria accounted for the largest proportion, followed by fungi, and gram-positive bacteria accounted for the smallest proportion. *Streptococcus*, *Aspergillus*, and *Pseudomonas aeruginosa* were the most common isolates. Gram-positive bacteria were most sensitive to vancomycin and susceptibility to imipenem showed the highest in gram-positive bacteria.

Traditional laboratory diagnosis of endophthalmitis Microscopic smear and culture results of intraocular fluid are of great significance for diagnosis and culture results are deemed the gold standard for clinical diagnosis and medication guidance[23]. However, only less than 1% of environmental microbes can be cultured with current techniques and in only 40% of cases can culture yield a putative pathogen result[24-25]. Moreover, whether a sample is positively-cultured is related to many factors, such as the clinical characteristics of the patient, the sampling sites as well as laboratory facilities. Studies indicate that both aqueous tap and vitrectomy can increase the possibility of obtaining a positive culture[26]. Culture media also poses influences to culture results[27-29]. We summarized the sequencing and culture results of clinically suspected endophthalmitis in Table 1[4,20,23,26,29-49]. Due to the low and varied culture rate and the small amount of intraocular fluid samples, rapid and accurate diagnosis of acute endophthalmitis is very important and closely related to the degree of visual loss of the patient.

Molecular diagnostic techniques of endophthalmitis Thereafter, a series of molecular biology methods emerged, providing new insights into the pathology of the ocular microbiome and ocular infections.

First came the technology of 16S polymerase chain reaction (PCR), which proves to be more accurate and shows more sensitivity in comparison with the traditional culture[46]. Afterwards, with the advent of an unbiased sequencing technique, the NGS of 16S rDNA amplicons and later the application of shotgun metagenomics, a more advanced and prompt method has been proposed for the detection of insidious pathogens in endophthalmitis. All pathogens, even those unculturable can be detected, without being limited by the starting primers[50-51]. Although NGS has the advantage of high throughput, its short-read characteristics render it difficult to identify repeats in gene sequences, followed by several other defects[52-54]. More recently, nanopore-targeted sequencing, characterized by long-read, also known as the fourth-generation sequencing technique, appears to be a promising real-time diagnostic platform for infectious endophthalmitis, especially in culture-negative cases[32-33].

Table 1 Culture results of intraocular fluid samples from endophthalmitis patients by different teams

Teams	Techniques	Sample capacity	Positive rate
Zhu *et al*[30] (2022)	Culture, mNGS	36	27.8%, 88.9%
Low *et al*[31] (2022)	Culture, Illumina WGS, 16S Nanopore, Nanopore WGS	23, 20, 18, 23	78.3%, 73.9%, 75%, 83.3%
Huang *et al*[32] (2021)	Culture, NTS	18	44.4%, 94.4%
Jun *et al*[33] (2021)	Culture (AH), Culture (VH), NanoAmpli-Seq (AH), NanoAmpli-Seq (VH)	8	37.5%, 75%, 100%, 75%
Mishra *et al*[34] (2021)	NGS, PCR	16	100%, 62.5%
Kosacki *et al*[35] (2020)	Culture, panbacterial PCR, culture & PCR	142, 137, 128	54.2%, 48.9%, 64.1%
Selva Pandiyan *et al*[36] (2020)	Culture, panbacterial PCR	88	19.3%, 34.1%
Xu *et al*[23] (2020)	Culture	44	45.5%
Feng *et al*[37] (2020)	Culture	157	45.0%
Bhikoo *et al*[26] (2020)	Culture	259	52.1%
Corredores *et al*[38] (2021)	Culture	16	62.5%
Mak *et al*[39] (2020)	Culture	18	27.8%
Zhou *et al*[40] (2020)	Culture	22	9.1%
Gandhi *et al*[4] (2019)	Culture, Illumina NGS	75	24%, 86.7%
Mishra *et al*[41] (2019)	Traditional culture, Automated culture, Broad-range PCR	195	8.7%, 30.8%, 65.1%
Yang *et al*[42] (2018)	Culture	670	39.7%
Xu *et al*[43] (2018)	Culture	40	60.0%
Deshmukh *et al*[44] (2019)	Culture Illumina NGS	34	44.1%, 88.2%
Deshmukh *et al*[45] (2018)	Culture	46	54.3%
Pongsachareonnont *et al*[29] (2017)	Plate culture, blood culture, PCR	41	12.2%, 26.8%, 26.8%
Sachdeva *et al*[20] (2016)	Culture	50	68.0%
Lee *et al*[46] (2015)	Culture, qPCR, Illumina NGS	21	66.7%, 47.6%, 57.1%
Gower *et al*[47] (2015)	Culture	502	58.0%
Pijl *et al*[48] (2010)	Culture	250	66.4%
Wong *et al*[49] (2004)	Culture	34	61.8%

mNGS: Metagenomic next-generation sequencing; qPCR: Real-time quantitative polymerase chain reaction; NTS: Nanopore targeted sequencing; AH: Aqueous humor; VH: Vitreous humor.

It is to be emphasized that traditional metagenomics, referred to as 16S metagenomics or targeted amplicon sequencing (TAS), aims to analyze the gene loci (such as 16S rRNA and ITSs[13]) of bacteria or fungi with specific characteristics through amplicon-based PCR targeted sequencing. Though accurate in identification, this method shows a number of limitations, such as the high requirements for amplicons and the relatively small number of species that can be detected due to the lack of reference taxonomy[55]. However, shotgun metagenomics or microbial whole-genome sequencing (MWGS), realized by the sequencing process of the fragmented DNA after being extracted directly from the environment and then sheared without isolation of microbes, enables high-throughput screening and detection of pathogens. Although this method reduces the resolution of presently-known pathogens (which targeted methods can better achieve), more detailed information can be obtained through this "untargeted" process, which is conducive to the detection of rare pathogens[56-58].

Metagenomic methods can provide taxonomic analysis of the microbiome, assess its potential function, and quantify it. The clinical applications include identifying microbial species, metabolic pathways, and metabolites related to the development and treatment of human diseases, and further promoting the discovery of microbiome-targeted drugs, and improving human health management efforts[34,59]. By simultaneously conducting NGS and culture on infected endophthalmitis and normal vitreous specimens, Deshmukh *et al*[44] concluded that the specificity of culture and clinical diagnoses of NGS was 20% and 100%, and the sensitivity of culture and clinical diagnoses of NGS was 87.5% and 88%, respectively. Therefore, NGS is expected to be a diagnostic platform for infective culture-negative endophthalmitis. In addition, by isolating culture-based microorganisms from patient specimens, whole genome sequencing (WGS) can be performed on these microorganisms, thus determining their taxonomic affiliation, phylogenetic relationships, potential antibiotic resistance genes, and virulence-related genes[60].

Meanwhile, long-read sequencing allows precise assembly of bacterial genomes in the complex microbiome and holds promise for the identification of undiscovered organisms[61]. Nanopore sequencing technology enables single-stranded DNA or RNA to pass through nano-sized holes in artificially manufactured membranes to convert biological signals of bases into current signals, which are then interpreted by algorithms

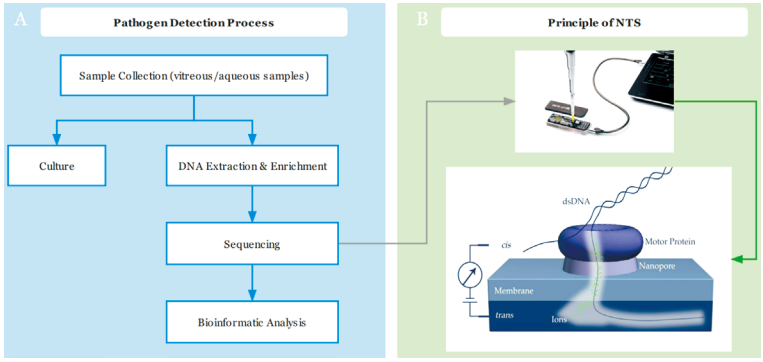

Figure 1 Procedure for the detection of intraocular fluid samples by MinION A: The process of ocular sample collection and detection; B: The principle of the NTS. dsDNA stands for double-stranded DNA. The *cis* side is negatively charged and the *trans* side is positively charged. NTS: Nanopore targeted sequencing.

and metagenomic matching can be performed[62] (Figure 1). Recently, the Oxford Nanopore MinION has been widely favored due to its portability, rapidity, and full-length 16S rRNA in data reading, exhibiting a good application prospect. Through the Oxford Nanopore sequencing technology, the 16S rRNA gene of bacteria was directly amplified by PCR, realizing rapid identification of bacteria[63]. Besides, several studies have demonstrated the capabilities of nanopore sequencers in bacterial metagenomic applications, especially in bacterial infections[64-66]. By using nanopore platforms for long-read deep sequencing, a cross-sectional diagnostic comparison study conducted by Low *et al*[31] testifies to show the potential of nanopore sequencing for cost-effective real-time identification of causative pathogens in endophthalmitis. Moreover, the technique could also be used to understand bacterial genomic properties related to virulence and antibiotic resistance[67].

Prophylaxis, Treatment, and Prognosis of Endophthalmitis

A retrospective, cross-sectional study conducted by Zafar *et al*[68] revealed a range of risk factors associated with the development of endophthalmitis within 90d after cataract surgery, which includes age, gender, race, and Charlson comorbidity index (CCI). Friling *et al*[69] stated that the risk of endophthalmitis after immediately sequential bilateral cataract surgery (ISBCS) is relatively lower than delayed sequential bilateral cataract surgery (DSBCS) but not to be neglected. However, later studies pointed out that there was no difference in the risk of postoperative endophthalmitis between bilateral and unilateral surgeries[70-71].

Preventive measures after cataract surgery are also associated with endophthalmitis[72]. Melega *et al*[73] verified in a randomized controlled trial that intracameral injection of 0.5% moxifloxacin reduced the incidence of endophthalmitis after cataract surgery. Intraocular lenses (IOLs) loaded with moxifloxacin (MXF) and ketorolac (KTL) were able to release antibiotics at therapeutic levels while displaying good biocompatibility[74]. A retrospective cohort study conducted by Ho *et al*[75] shed light on the conducive effect of early pars plana vitrectomy (PPV) on the prognosis of acute endophthalmitis after eye surgery and pointed out that endophthalmitis after post-cataract surgery as well as negative culture was associated with better prognosis. Meanwhile, endoscopic vitrectomy may allow for the early management of endophthalmitis, thereby controlling infection and avoiding evisceration[75-76].

Study shows that nanopores have revolutionized the prognosis and treatment of diseases by simply using femoler-scale analytes[77]. And in recent years, nanomaterial has shown advantages in the treatment of endophthalmitis. An *in situ* gel system with nanostructured lipid carriers (CIP-NLC-IG) was developed for topical ocular administration to enhance and sustain the antimicrobial activity of therapy of bacterial endophthalmitis[78]. Then came the photodynamic therapy. Chen *et al*[79] constructed ZIF-8-PAA-MB@AgNPs@Van-PEG, a composite nanomaterial that showed good biocompatibility and antibacterial ability. Ye *et al*[80] developed the AuAgCu$_2$O-bromfenac sodium nanoparticles (AuAgCu$_2$O-BS NPs), and with their photodynamic effects and nanostructures releasing metal ions and sodium bromfenac, antibacterial, anti-inflammatory, and Methicillin-resistant Staphylococcus aureus (MRSA) killing effects can be simultaneously realized, thus co-treating endophthalmitis after cataract surgery, which has been confirmed *in vivo* and *in vitro*. Later, Li *et al*[81] reported a cationic aggregation-induced luminescence of triphenylamine thiophene pyridinium (TTPy) for photodynamic treatment of bacterial endophthalmitis. TPPy has a good antibacterial effect in rat models, which triggers innate immune responses in the early stages of infection, limits the subsequent intense inflammatory response, and protects the retina from bacterial toxins and inflammation-induced bystander injury effects. It preserves vision, giving TTPy potential for clinical application in ophthalmic infections.

It is to be noted that the identification of causative pathogens is of great significance for the prognosis of endophthalmitis.

Pathogens with low virulence have a better prognosis prone to show a better prognosis. For example, the coagulase-negative staphylococcal endophthalmitis had a better prognosis than streptococcal endophthalmitis[1]. Kirstahler *et al*[60] studied the application of NGS technology supported by WGS in the disease management of endophthalmitis after cataract or intravitreal injection, pointing out that this technology may better distinguish infectious from sterile endophthalmitis. In the case of a very high coverage depth of metagenomic sequencing, it can also reveal valuable functional information, such as antibiotic resistance and virulence-related genes[82].

DISCUSSION

The microbe of the ocular surface is closely related to the occurrence of postoperative endophthalmitis[83]. The use of metagenomics to obtain information related to the bacteriocin gene of ocular microbe may better guide the treatment of endophthalmitis. Owing to the narrow range of activity many bacteriocins have compared to most existing antibiotics, they hold great promise for precise treatment and prevention of infection[84]. In addition, metagenomics is of great importance in the surveillance of suspected microorganisms that may cause disease[85]. By the way, anti-microbial resistance is also a problem that cannot be ignored, and metagenomic methods can enable us to better understand and control it.

However, nanopore sequencing has some drawbacks to be improved. For instance, its application of 16S sequencing is limited to bacterial identification[86]. The hypervariable region of 16S rRNA is deemed the standard bar code for bacteria, while the internal transcription spacer 1 (ITS1) of the ribosomal RNA gene cluster has a high potential for identifying eukaryotes. Also, the Meta-barcode data analysis relies on a carefully managed barcode reference resource. Santamaria *et al*[87] created ITSoneDB in order to produce a comprehensive set of ITS1 sequences with robust taxonomies. More such references need to be clarified. In addition, long-read sequencing assembly can have a range of errors. To further address this problem, Wick *et al*[88] introduced a tool called Trycyler that makes the results more accurate than in the case of automatic assembly. Woyke *et al*[89] proposed that the metagenomic assembly and the assembly-free methods can be combined in reads analysis after sequencing to complement and verify each other. Other drawbacks include the high cost of testing, high requirements for starting materials, and the need for a specific analysis of various steps[90]. Finally, and fundamentally, metagenomics cannot solve certain problems that only pure culture can solve. For example, with genomic information alone, we cannot further analyze and identify the processes related to cellular physiology in the causative microorganisms, which are closely related to microbial metabolism and host pathogenic processes[91].

Meanwhile, the possibility of applying ribosome analysis to uncultured mixed communities is being explored[92]. In a prospective multicenter study diagnostic evaluation study, vitreous samples from acute or delayed-onset postoperative endophthalmitis patients were analyzed using combined methods including bacterial cultures in pediatric blood culture bottles and panbacterial PCR, which provides a new idea for rapid diagnosis of causative pathogens[35].

To date, given the technological innovation especially the NTS technology, which has ushered in a new era, the diagnosis and treatment of endophthalmitis have made great progress from a clinical standpoint. Moreover, the application of metagenomic technology and nanopore sequencing in endophthalmitis is also expanding. Automated sequencing analysis platforms based on bioinformatics technology are also being developed to make the analysis of sequencing data more accurate[93-95].

In this review, we briefly introduced the current research status of molecular sequencing in endophthalmitis and discussed exciting development in sequencing technology. Despite the challenges, we believe that nanopore-targeted sequencing has greatly contributed to the progress of genomics in the field of endophthalmitis and that this is the way forward for research. It is likely that we will see an increasing number of clinical trials testing combinations of technologies, probing potential mechanisms, and collaborating to drive progression in the diagnosis and treatment of endophthalmitis.

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