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Comparative evaluation of 16S rRNA metagenomic sequencing in the diagnosis and understanding of bacterial endophthalmitis

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ABSTRACT

Objective To evaluate the usefulness of metagenomic analysis in the search for causative organisms of bacterial endophthalmitis.

Methods and analysis Twenty-one consecutive treatment-naïve patients (13 men and 8 women; mean age, 60.8±19.8 years) with suspected endophthalmitis were recruited. Vitrectomy was performed to diagnose and treat endophthalmitis. Bacterial culture and metagenomic analysis of the vitreous body were performed. Extracted DNA was analysed using 16S rRNA sequences, and libraries were sequenced on an Illumina MiSeq sequencer. To compare the bacterial composition in each case, α and β diversities were determined.

Results Patients were categorised into three groups: endophthalmitis cases with matching predominant organisms according to metagenomic analysis and bacterial culture, those with negative results for bacterial culture and those with negative results in both cases. In 7 of 15 culture-negative cases, results from metagenomic analysis could detect pathogens. The diversity of bacterial populations was significantly lower in the group with positive results for predominant bacteria according to culture and metagenomic analysis. All patients with uveitis were included in the group for which the causative pathogen could not be determined by culture or metagenomic analysis. The structures of bacterial populations significantly differed between the positive and negative groups by culture and metagenomic analysis.

Conclusions Metagenomic analysis could be useful for prompt detection of causative pathogens, for precise diagnosis of infection, and as a marker of inflammation processes such as uveitis.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Traditional endophthalmitis diagnosis employs ocular fluid culture and PCR, usually providing reliable results. However, the differentiation between infection and inflammation is sometimes difficult with these methods, particularly in cases with a negative result from the bacterial culture, warranting the exploration of more comprehensive diagnostic approaches.

WHAT THIS STUDY ADDS

⇒ Metagenomic analysis successfully detected the causative pathogen in endophthalmitis cases with both positive and negative results from the bacterial culture. A rich α diversity was observed in uveitis cases lacking a predominant bacterial species, indicating a complex and balanced microbial community.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Metagenomic analysis in bacterial endophthalmitis enables detailed detection independently of bacterial culture results, allowing for precise infection diagnosis and informing appropriate antibiotic selection. This method also differentiates between infection and uveitis by assessing bacterial diversity.

INTRODUCTION

Endophthalmitis is a severe infection in the eye that can occur as a consequence of intraocular surgery, intraocular injections, trauma, the presence of a central venous catheter and systemic infectious diseases such as sepsis, abscesses or urinary tract infection.[1 2] Acute bacterial endophthalmitis represents a significant ocular pathology that could potentially lead to blindness, requiring prompt and accurate diagnosis followed by a suitable therapeutic intervention.[3] Coagulase-negative *Staphylococcus* has been identified as the predominant organism implicated in cases of endophthalmitis following intravitreal injections or surgical procedures.[4] In some cases, Gram-negative bacteria such as bacilli and *Pseudomonas* were also isolated.[3 4] Standard methods to determine pathogens include bacterial culture and PCR using an ocular fluid such as aqueous or vitreous humour as sample; however, these techniques have limitations in terms of accurate pathogen identification.[1–3] In cases of suspected endophthalmitis, cultures from aqueous and

vitreous humour samples show a positivity rate of approximately 30%, whereas that for PCR ranges from 46% to 60%.[1–3] The low sensitivity of microbiological cultures is due to factors such as small sample volumes, a small specimen numbers and the differential culture conditions for each strain.[1–4] While PCR can detect bacterial presence via 16S rRNA analysis, it is still limited in accurately identifying specific bacterial species, underscoring the need for more refined techniques for this purpose.[1–3] Accurate identification at the genus or species level is crucial for accurate diagnosis and appropriate treatment.[1–3] Misidentification of the causative bacterial species prevents the administration of suitable antimicrobial treatments.[1–4] Consequently, there is an urgent need to develop a diagnostic approach that is both fast and accurate.

Clinically, whole genome sequencing (WGS) and metagenomic analysis have been performed as diagnostic approaches.[5] In postprocedure endophthalmitis, higher load of bacteria from WGS has worse visual prognosis.[6] While WGS aims to analyse the genome of a single bacterium, 16S rRNA metagenomic analyses and shotgun metagenomics are used to comprehensively determine bacterial compositions in clinical specimens.[7–10] Metagenomic analysis effectively detects pathogens responsible for systemic infectious diseases such as sepsis, pneumonia and urinary tract infection; in a short period of time and with high positivity rates.[11–13] If pathogens are detected in the early stage of sepsis, proper selection of antibiotics for treatment can reduce the mortality rate.[11–13] There are few reports on the use of metagenomic analysis to identify causative pathogens of ocular infectious diseases from intraocular samples.[1–6] Therapeutic intervention is required in cases of endophthalmitis, and analysis of the collected samples is considered to be useful for determining treatment strategies.[1–4] In cases of this syndrome, vitreous body surgery and metagenomic analysis are useful not only for treatment but also for the identification of causative pathogens.[1–4] The purpose of this study was to evaluate the usefulness of metagenomic analysis for determining the causative pathogens in endophthalmitis.

MATERIALS AND METHODS

Patients and sampling

In this study, 21 vitreous body samples from 21 consecutive treatment-naïve patients (13 men and 8 women; mean age, 60.8±19.8 years) with suspected endophthalmitis were collected at Osaka University Hospital and Tokyo Medical University Hospital from April 2020 to January 2022. Clinical data and background information of each patient are presented in online supplemental table 1. Sample collections were performed in a clean operation room. Vitreous samples were collected immediately after inserting a vitreous cutter (Alcon Laboratories, Geneva, Switzerland) into the vitreous cavity through sclerotomy and performed without intraocular irrigation. Samples were carefully transferred into DNA LoBind tubes (Eppendorf, Fremont, California). Two types of samples from each patient were obtained for bacterial culture and metagenomic analysis.

Samples for metagenomic analysis were promptly stored at −80°C until DNA extraction, and 500 µL of the vitreous body was used for the procedure. DNA was extracted from each sample using a Power Soil DNA Isolation Kit (MoBio, Carlsbad, Californi) according to the manufacturer's instructions. The extracted genomic DNA was eluted in 100 µl of the kit elution buffer and stored at −20°C until analysis.

In this study, negative controls using distilled water (DW) were included for microbiome comparison.

Metagenomic analysis

Metagenomic analyses were performed via 16S rRNA sequencing. Each library was prepared according to the 'Illumina 16S Metagenomic Sequencing Library Preparation Guide', with a primer set (27Fmod: 5′-AGR GTT TGA TCM TGG CTC AG-3′ and 338R: 5′-TGC TGC CTC CCG TAG GAG T-3′) targeting the V1–V2 region of the 16S rRNA gene. Then, 251 bp paired end sequencing of the amplicon was performed on a MiSeq (Illumina) using an MiSeq v2 500 cycle kit (first PCR: 15 cycles and second PCR: 15 cycles[14]). The paired end sequences obtained were merged, filtered and denoised using DADA2. Taxonomic assignment was performed using QIIME2 feature classifier plugin with the Greengenes 13_8 database.[15] The bioinformatics pipeline QIIME2 (V.2020.2) was used as the informatics environment for all the processing of raw sequencing data. To visualise the positions of each group, the Emperor software tool was used for principal component analysis (PCA).[16]

For identification of the bacterial flora via metagenomic analysis, a positive result was defined as the predominance of a specific bacterial population.

Statistical analysis

Statistical analyses were performed using JMP software V.17.0 (SAS, Cary, North Carolina) and the R software environment (in the public domain, http://cran.r-project.org/). To compare α and β diversity, Kruskal-Wallis tests and permutation analysis of variance (PERMANOVA) were performed, respectively. The chi-square tests and Wilcoxon rank-sum tests were used to identify significant differences, using a significance threshold of $p<0.05$.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting or dissemination plans of this research.

RESULTS

Clinical information and classification of patients

Twenty-one consecutive treatment-naïve patients with suspected endophthalmitis participated in the study. The participants were allocated into the following three groups: endophthalmitis cases with matching predominant organisms according to metagenomic analysis

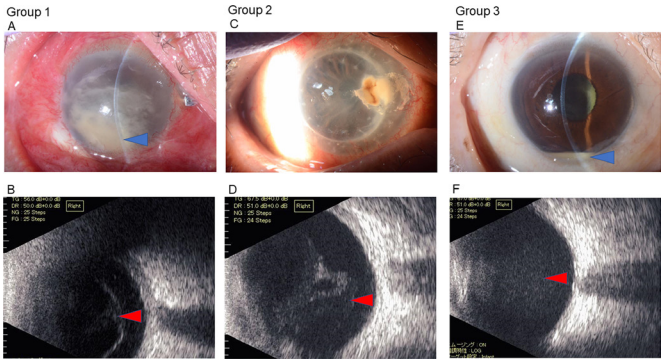

Figure 1 Representative clinical features from the three groups of patients. In all cases, severe inflammation such as hyperaemia and hypopyon (blue arrowheads) at the anterior segment and dense vitreous opacity (red arrowheads) in the vitreous body on B-scan ultrasonography were present. (A, B) Group 1: Cases with positive results for bacterial culture and metagenomic analysis. (C, D) Group 2: Cases with positive results for metagenomic analysis and negative results from bacterial culture. (E, F) Group 3: Cases with negative results for both methods of diagnosis.

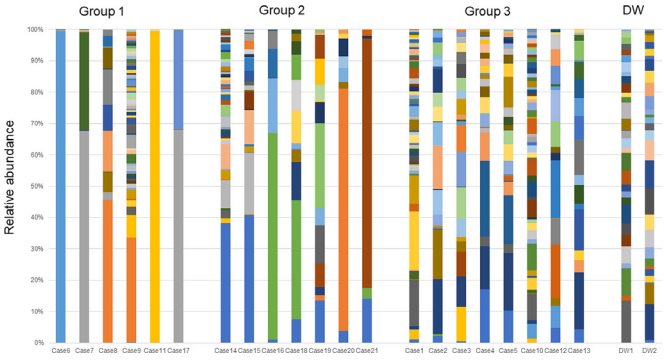

Figure 2 Comparison of bacterial populations and species composition in each sample. There are three test and negative controls groups (group 1: cases with positive results for metagenomic analysis and bacterial culture, group 2: cases with positive results for metagenomic analysis but negative results for bacterial culture negative and group 3: cases with negative results for metagenomic analysis and bacterial culture; distilled water (DW) used as negative controls). The same colour represents the same bacterial population. In group 1, a statistical decrease in α diversity can be observed, with two cases exhibiting an occupancy rate close to 100%, and the remaining four cases showing rates above 30%. In group 2, a statistical decrease in diversity and an occupancy rate above 25% can be observed. In group 3, the bacterial populations are diverse, and the occupancy rate is never above 25%. In negative controls, diverse bacterial populations are observed, and the bacterial occupancy rate is below 15% in all cases.

and bacterial culture (group 1; six patients), cases with negative results for the bacterial culture (group 2; seven patients) and cases in which the predominant bacterial species could not be identified by either method (group 3, eight patients). All patients with a history of uveitis (five) were included in group 3. Detailed information on the patient characteristics is presented in online supplemental table 1.

Clinical findings in each group

Clinical features of representative cases for each group are shown in figure 1. The positivity rates for metagenomic analysis and culture were 61.9% (13 out of 21) and 28.5% (6 out of 21), respectively. There was a statistically significant difference between both rates (Chi-square; p=0.0299). In 7 of 15 culture-negative cases, results from the metagenomic analysis could detect pathogens.

Staphylococcus epidermidis, *Klebsiella pneumoniae*, *S. aureus* and *Streptococcus mitis* were detected by bacterial culture in group 1. In all cases (6, 7, 8, 9, 11 and 17), metagenomic analysis yielded the same results. In case 11, slit lamp examination showed conjunctival infection (hypopyon within the anterior chamber). B-scan ultrasonography showed dense vitreous opacity (figure 1A,B).

The presence of *Pseudomonas veronii*, *Bradyrhizobium japonicum*, *Methylobacterium mesophilicum*, *S. aureus* and *M. adhaesivum* was identified by metagenomic analysis in the seven patients included in group 2 (cases 14, 15, 16, 18, 19, 20 and 21). In case 14, slit lamp examination showed conjunctival infection, corneal oedema and corneal infiltrate in the nasal area postkeratoplasty suture. B-scan ultrasonography showed diffuse vitreous opacity (figure 1C,D).

Cases 1, 2, 3, 4, 5, 10, 12 and 13 did not yield positive results for either identification method. In case 13, slit lamp examination showed hypopyon within the anterior chamber, and B-scan ultrasonography showed slight vitreous opacity (figure 1E,F).

DNA sequencing in each group and comparison

The total reads for each sample are described in online supplemental table 1. In this study, 32.4% of reads were assigned to the species level, whereas 73.0% were assigned to the genus level. The mean read from groups 1, 2, 3, and negative controls were 67 883±22 155, 72 938±32 241, 70 105±22 577 and 35 081±13 126, respectively. Statistically significant differences were observed between group 1 and negative controls (p=0.0428), group 2 and negative controls (p=0.0472) and group 3 and negative controls (p=0.0219). No significant differences were observed between group 1 and group 2 (p=0.943), group 1 and group 3 (p=0.746) and group 2 and group 3 (p=0.862).

Relative abundance and composition of the bacterial flora

The metagenomic analysis results for all participants in the study are presented in figure 2. In the analysis of populations at the species level, the isolated bacteria from group 1 were *S. epidermidis* in 99.7% of case 6, *K. pneumoniae* in two cases (67.5% of case 7 and 68% of case 17), *S. aureus* in two cases (45.7% of case 8 and 31.1% of case 9) and *S. mitis* in 99.4% of case 11. In group 2, the identified pathogens were *P. veronii* in two cases (38.1% of case 14 and 40.6% of case 15), *B. japonicum* in two cases (66.0% of case 16 and 38.0% of case 18), *M. mesophilicum*

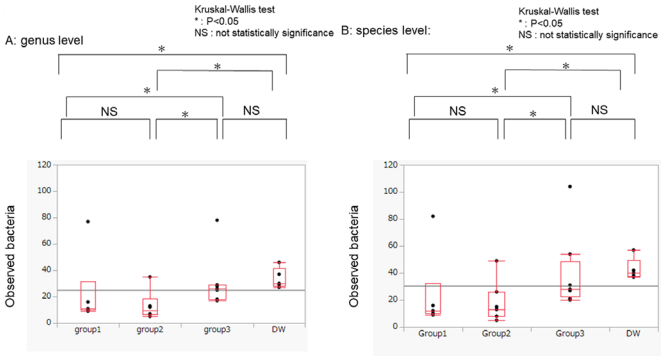

Figure 3 Comparisons of bacterial α diversity at the genus level (A) and species level (B) among the four groups (group 1: cases with positive results for bacterial culture and metagenomic analysis, group 2: cases with positive results for metagenomic analysis but negative results for bacterial culture and group 3: cases with negative results for metagenomic analysis and culture); distilled water (DW) used as negative controls. At the genus level and species level both, similar results are obtained. The number of observed bacterial species was determined, and the results were compared using the Kruskal-Wallis test. The α diversity in groups 1 and 2 was lower compared with that in group 3. There were statistically significant differences between group 1 and group 3 ($p<0.05$), and between group 2 and group 3 ($p<0.05$). There was no significant difference between group 1 and group 2 ($p>0.05$). Statistically significant differences are observed between group 1 and negative controls ($p<0.05$) and between group 2 and negative controls ($p<0.05$). Conversely, no statistically significant differences are observed between group 3 and negative controls ($p>0.05$).

in 27.0% of case 19, *S. aureus* in 77.2% of case 20 and *M. adhaesivum* in 79.5% of case 21.

In negative controls using DW, diverse range of bacterial populations, with no dominant populations were observed. In addition, all the bacterial occupancy rate was below 15%. Compared with the negative control, groups 1 and 2 exhibited dominant populations, and the percentage of most dominant population was more than double that of second dominant population.

Bacterial richness according to α diversity

Bacterial α diversity among the three groups of patients with suspected endophthalmitis was investigated at the genus and species level and the results are shown in figure 3A,B, respectively.

At the genus level (figure 3A), the number of bacteria varied across the group, with group 1 having 22.3±8.25 (range: 9–77) bacteria, group 2 having 13.1±8.25 (range: 5–35) bacteria, group 3 having 31.5±7.60 (range: 17–78) bacteria and negative controls having 33.8±8.44 (range: 27–46) bacteria. Statistically significant differences were observed between groups 1 and 3 (p=0.0381) and between groups 2 and 3 (p=0.0381). In groups 1 and 2, the number of bacteria was lower compared with that in group 3. However, no significant difference was observed between groups 1 and 2 (p=0.469). Statistically significant differences were observed between group 1 and negative controls (p=0.0450) and group 2 and negative controls (p=0.020). In contrast, no statistically significant differences were observed between group 3 and negative controls (p=0.0629).

At the species level (figure 3B), the number of bacterial species discriminated by group was 23.5±11.7 (range: 9–82) in group 1, 17.7±5.82 (range: 5–49) in group 2, 39.1±9.98 (range: 20–104) in group 3 and 42.8±10.18 (range: 37–57) in negative control. There were statistically significant differences between group 1 and group 3 (p=0.0381) and between group 2 and group 3 (p=0.0381). In groups 1 and 2, the number of bacterial species was lower compared with that in group 3. There was no significant difference between group 1 and group 2 (p=0.469). Statistically significant differences were observed between group 1 and negative controls (p=0.0381) and group 2 and negative controls (p=0.0183). In contrast, no statistically significant differences were observed between group 3 and negative control (p=0.141).

Bacterial similarities with PCA analysis and β diversities

In PCA, the first two principal components capture 17.9% (PCA1) and 12.5% (PCA2) of variance, respectively. Principal components represent the different directions of each group (figure 4A). In this analysis, the similarities of four groups (group 1: red, group 2: blue, group 3: orange and negative controls: green) were indicated by the distances between each location.

The β diversity distances between the four groups are shown in figure 4B. β diversity was investigated using PERMANOVA. There were significant differences in bacterial composition between group 1 and group 3 ($p<0.001$) and between group 2 and group 3 ($p<0.001$). In contrast, there were no significant differences in bacterial composition between group 1 and group 2 (p=0.363). Statistically significant differences were observed between group 1 and negative controls ($p<0.001$) and between group 2 and negative controls ($p<0.001$). Conversely, no statistically significant differences were observed between group 3 and negative controls (p=0.467).

DISCUSSION

We propose metagenomic analysis as a quick and accurate diagnostic method for detecting pathogens in bacterial endophthalmitis. We confirmed that the culture-positive results from bacterial cultures matched those from metagenomic analysis in terms of pathogen identity. We also found that causative pathogens were detected by metagenomic analysis even in cases in which bacterial cultures had yielded no results. Patients with a history of uveitis did not exhibit positive results from either of these methods. Confirmation of α and β diversities is both helpful to differentiate between cases involving infection or not. In the cases of endophthalmitis, α diversity was decreased and no bacterial occupancy rate above 25% was observed. The β diversity, which indicates similarity, was different between cases involving infection

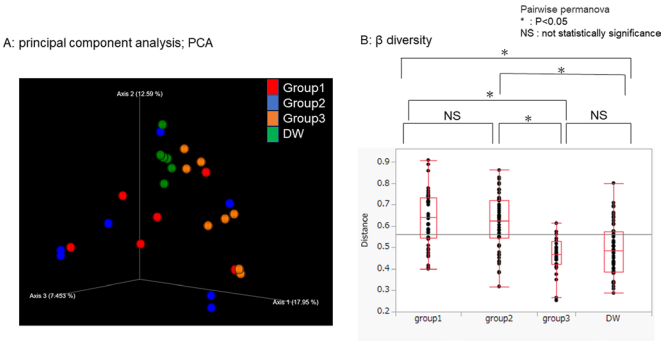

Figure 4 Bacterial similarities according to principal component analysis (PCA) and β diversities. There were four groups (group 1: cases with positive results for metagenomic analysis and bacterial culture, group 2: cases with positive results for metagenomic analysis positive but negative results for bacterial culture, group 3: cases with negative results for metagenomic analysis and bacterial culture, and distilled water (DW) used as negative controls. (A) In the PCA analysis, the similarities of bacterial groups (group 1: red, group 2: blue, group 3: orange, and negative controls: orange) are indicated by the distances between each location. The PCA analysis revealed a separation between the four groups. (B) β diversity was estimated using permutation analysis of variance (PERMANOVA). Significant differences were detected between group 1 and group 3 ($p<0.001$), and between group 2 and group 3 ($p<0.001$). There was no significant difference between group 1 and group 2 ($p>0.05$). Statistically significant differences are observed between group 1 and negative controls ($p<0.001$) and between group 2 and negative controls ($p<0.001$). In contrast, no statistically significant differences are observed between group 3 and negative controls ($p>0.05$).

and those that did not. Metagenomic analysis can detect the pathogen within a few days, allowing for appropriate selection of antibiotic treatment and the subsequent preservation of visual function.

Metagenomic analysis enables the analysis of DNA segments from multiple microorganisms without the need for culture and is carried out using either an amplicon or shotgun-based approach.[17] Previously, short-read sequencing of the bacterial 16S rRNA gene has been the standard method for microbiome profiling; however, recently long-read sequencing method has been employed to achieve a more comprehensive microbiome profiling.[18] Long-read methods offer higher detection confidence than short-read methods do. Moreover, long-read methods provide strain-level community detection.[19] Although long read sequence had not been performed in this study, we analysed the V1–V2 region based on a previous report because of the good performance.[14] Both short-read and long-read metagenomic methods have yielded similar results in detecting similar bacterial compositions.[14] Considering, the target disease in this study was endophthalmitis, which is an emergency disease,[20] the most important factor was the timely detection time for prompt treatment while complete identification of the bacterial strain is desirable.

Detecting bacterial pathogens in endophthalmitis based on bacterial culture poses significant challenges due to specific growth requirements.[11–13] In cases of postoperative endophthalmitis, Gram-negative bacteria were identified as the infecting pathogen in almost 50% of the cases, whereas Gram-positive bacteria were present instead in 20% of the cases, and no pathogen was identified in the remaining cases.[21] In previous studies, the causative bacterial species varied according to the endophthalmitis type. Notably, bacterial agents could not be identified in numerous cases, highlighting diagnostic challenges.[22] Metagenomic analysis makes it possible to detect pathogenic bacteria at the species level.[23 24] Previous reports have shown that a 16S rRNA metagenomic analysis encompasses the whole genomic DNA present in the sample, leading to potential issues with contamination.[25] As a result, indigenous bacteria, such as *Methylobacterium* and *Corynebacterium,* have often been identified in experiments and reported as contamination.[26] In this study, negative controls were included, and various types of commensal bacteria were detected. The occupancy rate of these bacteria was very low (<0.05%) and no bacteria suspected to be causative agents were identified. Additionally, if viruses or fungal infection occurred, the number of reads associated with these pathogens could be higher than that of the bacterial species. In addition, due to the presence of specific viruses or fungi in the sample, the predominance of bacterial species may not be accurately detected. Our findings also demonstrate that the pathogenesis for the syndrome can be determined even in cases with negative results from the bacterial culture. In fact, even if endophthalmitis is suspected clinically and the culture yields negative results, treatment for endophthalmitis is often continued until clinical findings improve.[11–13 21] If the metagenome yields positive results in culture-negative cases, it can serve as a basis for continuing the use of antibiotics.

It is difficult to clinically determine in which cases infection is present.[27–29] This study demonstrates alterations in α and β diversities in endophthalmitis. Considering a 25% occupancy rate as threshold, our results may provide a valuable diagnostic tool for inflammatory ocular conditions such as uveitis. In the patients diagnosed with uveitis, both culture and metagenomic analyses yielded negative results. Interestingly, α diversity was preserved, and no bacterial populations surpassed a 25% occupancy rate. In addition, in the negative controls, all the bacterial occupation rate was below 15% and the average diversity was similar to that of the non-infectious group. These results indicated that the adulteration of genomic remnants from the blood stream of uveitis or contamination may occur during the extraction process. Even if the pathogenic bacteria are present in the vitreous body, their pathogenicity may be low as long as the occupancy rate does not exceed the threshold. While case-specific factors may influence the selected cut-off value, our findings suggest that a decrease in diversity, coupled with

an evaluation of bacterial occupancy rates, can serve as potential indicators for the diagnosis of endophthalmitis. Although β-diversity is affected by causative pathogens, it is possible to diagnose endophthalmitis by combining different factors, including compositional differences, occupancy rate of major causative bacteria and decreased α-diversity.

This study had several limitations including its small sample size, which was a direct consequence of the low incidence of endophthalmitis, the small amount of vitreous body available may have resulted in some bacteria being missed during the identification. In the analysis, microbial composition in the vitreous body could have been affected by factors including patient demographics such as age, immunocompromised host and medication. In addition, the occupancy of specific bacteria may differ between the onset and advanced stages of endophthalmitis. Moreover, it was difficult to maintain the same condition across all patients since the reasons for developing infectious endophthalmitis were different for each individual. Regarding technical aspects of metagenomic sequences, taxonomic classification was limited to what was included in reference library, and results could have been affected by contamination and bias. Therefore, negative controls were essential to ensure the integrity and reproducibility of the results. Despite these limitations, the pathogens identified by the metagenomic analysis were consistent with those identified by culture results at the genus and species level.

In summary, metagenomic analysis offers expedited pathogen detection and allows for the discrimination between infectious and non-infectious ocular conditions, particularly in cases with negative results from bacterial culture. This approach facilitates swift pathogen detection, enables precise diagnosis and selection of suitable antibiotic treatment and allows differential diagnosis of uveitis cases.

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Contributors KA, NH, KM and KN planned the experiments; KA, NH, KM and DM performed the research and acquired data; KA, NH and KM analysed the data and wrote the Methods and Results sections of the manuscript; YU, SN and KN supervised the project; KA, NH, KM, TT and KN wrote and edited the manuscript. All authors contributed to the critical revision of the manuscript. NH was responsible for the overall content.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval The current study adhered to the tenets of the Declaration of Helsinki, the local ethics committee of the Osaka University Medical Hospital approved the study (approval ID 20253), and written informed consent was obtained from all subjects. Participants gave informed consent to participate in the study before taking part.

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Supplemental Table 1: Clinical data and background of each patient

Case	Age Range	Sex	Laterality	Past history	logMAR VA	Intra ocular pressure (mmHg)	Bacterial culture	Microbiome results	Group	Total Reads	Coverage (%)
1	55-60	F	Left	uveitis, atopic dermatitis	-0.08	19	–	–	III	87,355	–
2	60-65	M	Left	post-glaucoma surgery	1.2	12	–	–	III	48,393	–
3	70-75	F	Left	sepsis	2	15	–	–	III	40,505	–
4	80-85	F	Right	uveitis	2	11	–	–	III	67,000	–
5	20-25	M	Right	uveitis	2	11	–	–	III	64,511	–
6	75-80	M	Right	post-cataract surgery	1.4	7	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*	I	94,147	99.7
7	70-75	F	Right	sepsis, type1DM	2	15	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	I	82,462	67.6
8	60-65	M	Left	post-cataract surgery	2	18	*Staphylococcus aureus*	*Staphylococcus aureus*	I	58,357	45.7
9	60-65	M	Right	chronic kidney disease, HD	LP	29	*Staphylococcus aureus*	*Staphylococcus aureus*	I	47,774	33.4
10	35-40	M	Right	trauma	1.5	8	–	–	III	59,400	
11	70-75	F	Right	post-glaucoma surgery	NLP	26	*Streptococcus mitis*	*Streptococcus mitis*	I	39,985	99.4
12	40-45	F	Right	scleritis	2	6	–	–	III	109,838	–
13	70-75	F	Right	uveitis	2	12	–	–	III	83,841	–
14	75-80	M	Right	post-penetrating keratoplasty, cytomegalovirus infection	1.4	22	–	*Pseudomonas veronii*	II	69,739	38.1
15	50-55	F	Right	post cataract surgery	0	10	–	*Pseudomonas veronii*	II	93,399	40.6
16	75-80	M	Right	post-cataract surgery	0.52	22	–	*Bradyrhizobium japonicum*	II	52,606	65.9

17	45-50	M	Right	prostatic abscess	2	19	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	I	64,830	68.0
18	55-60	M	Left	post-cataract surgery, DM	0.7	24	–	*Bradyrhizobium japonicum*	II	32,264	37.9
19	70-75	M	Left	Orbital cellulitis, DM	LP	34	–	*Methylobacterium mesophilicum*	II	69,739	27.0
20	85-90	M	Right	prostate cancer	LP	30	–	*Staphylococcus aureus*	II	133,000	77.2
21	10-15	M	Right	trauma	-0.08	16	–	*Methylobacterium adhaesivum*	II	64,731	79.5

M = male; F = female; logMAR = logarithm of the minimum angle of resolution; VA = visual acuity LP = light perception; NLP = no light perception; HD = Hemodiafiltration; DM = Diabetes mellitus