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Recent Developments in Microbiological Methods for Pathogen Identification in Infectious Endophthalmitis

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Abstract

The medical emergency of infectious endophthalmitis necessitates an immediate clinical diagnosis and the start of treatment. Precision in the management of endophthalmitis is still difficult to achieve. We give an updated summary of recent research that reflect the most recent developments in clinical microbiological methods for infectious endophthalmitis in this review.

Keywords: Ophthalmology • Endophthalmitis • Retina • Microbiology • MALDI-TOF MS • PCR • Genome • Sequencing • Precision medicine • Infectious diseases

Introduction

An eye emergency called infectious endophthalmitis can cause severe, irreversible vision loss within hours or days following a clinical diagnosis. An infection of the intraocular vitreous and/or aqueous fluid caused by a causal agent, such as bacteria or fungi, is referred to as endophthalmitis. The posterior section of the orbit, between the lens and retina, contains the biggest component of the eye, known as the vitreous humour, which is made up of a mucilaginous, achromatic, highly hydrated matrix. The ciliary body secretes the watery, transparent fluid known as aqueous humour, which is found in the anterior and posterior chambers of the eye and leaves the body through the trabecular meshwork at the iridocorneal angle. Intraocular surgery, intraocular injections, trauma, contiguous spread from neighbouring structures (such as keratitis, bleb), and endogenous spread from chronic and sporadic sources from the bloodstream, such as a liver abscess or indwelling central venous catheter, respectively, can all lead to infection of the intraocular compartments [1-4].

The amount of the pathogen's inoculum, virulence factors, and immunological activity can all affect how endophthalmitis develops. Endophthalmitis risk can be increased by a significant number of microorganisms being introduced to the conjunctiva of the eye, such as contaminated surgical fluid. Membrane-damaging virulence agents, such as hemolysins, phospholipases, and sphingomyelinases, can advance invasion, causing retinal cell injury, an increase in inflammation, and ultimately irreversible vision loss. Therefore, it's important to understand the To preserve eyesight, a kind of endophthalmitis requiring quicker, more accurate diagnostic techniques with small sample numbers is essential. Endophthalmitis can occur for a variety of reasons, including post-cataract, post-vitrectomy, post-keratoplasty, post-injection, posttraumatic, bleb-related, keratitis-related, mould, and endogenous (bacterial, fungal) endophthalmitis, which have all been documented over the past few decades. Acute post-cataract endophthalmitis is a common kind of endophthalmitis. This bacterial type typically appears a week after cataract surgery. Infections frequently originate from the ocular surface or the flora of the lid skin. However, on occasion, infected surgical tools or solutions can be the cause of outbreaks, as demonstrated in a study examining postoperative Fusarium oxysporum endophthalmitis following the use of contaminated viscoelastic materials.

Can PCR survive when cultures fail using nucleotide-based methods?

The clinical movement toward "precision medicine," particularly in the field of infectious diseases, makes use of the enormous strides in genomic technology to define infections quickly and direct therapy and prevention. Infectious endophthalmitis is a disease that is studied in the papers below to determine the value of genome-centralized PCR (polymerase chain reaction) techniques for pathogen detection. This investigation includes 284 intraocular samples from 153 patients with delayed onset postoperative endophthalmitis (aqueous taps of 150–200 L, vitreous taps of 200–300 L, and vitrectomy biopsies of 500 L). Samples were inoculated in paediatric blood culture bottles for 14 days in order to conduct analysis using conventional microbiological culture, and they were then plated for phenotypic identification. Samples with enough culture-remaining volume were processed for DNA extraction and used for PCR analysis.

The frequencies of successful pathogen identification from samples examined before intravitreal antibiotics were: 77/142 (54%) with standard culture, 67/137 (49%) with 16S rRNA PCR, and 8/120 (7%), with qPCR. 6/25 A total of 24% of cases without a positive culture had 16S rRNA findings. The rates of effective pathogen removal from samples after intravitreal antibiotics (vancomycin and ceftazidime) 45/124 (36%) of the samples were successfully identified using standard culture, while 57/120 (16S rRNA PCR) were. The results of the qPCR assays showed that there was no discernible difference between the bacterial loads of the samples before and after intravitreal antibiotics. The 16S rRNA panbacterial PCR was shown to have a slower turnaround time and worse sensitivity and specificity than qPCR testing.

Results from the 16S rRNA panbacterial PCR took 2-3 days to process, whereas those from the qPCR took 2-3 hours. Due to the first stage of growth in blood culture bottles, the standard microbiological culture took longer than 14 days to complete. The microbiological profile of the samples and the prognosis for vision were not significantly correlated in this investigation, however a higher bacterial load in vitreous fluid samples was linked to a worse prognosis for vision. The Findings from this study by Kosacki indicate that 16S rRNA PCR may enable positive pathogen identification in the event of negative culture results. *S. epidermidis* accounted for 65% of the pathogens that were identified, but the study neglects to note whether the various techniques consistently identified the same pathogens. Small sample volume is a significant drawback in intraocular fluid microbiological research, which is also addressed in this study. Small endophthalmitis samples may benefit from

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the refinement of the method of incubating intraocular samples in blood culture bottles, allowing for a higher output of pathogen in more abundant proportions for further analysis with nucleotide-based or phenotype-based studies for identification. This increased sample production might make it possible to obtain antibiotic susceptibility profiles from the same sample source.

Using proteomic fingerprints to identify the offending pathogen with MALDI-TOF MS

The mass-to-charge properties of a pathogen's peptide components are used in Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) to create a proteomic profile of the pathogen and match the sample against a proteomic "fingerprint" database of a variety of organisms. To identify a pathogen to the species level, the MALDI-TOF MS laser ionises whole cell extracts from colonies grown in culture to yield a peptide fingerprint profile, which is then compared against a proteome database. This approach of proteomic "fingerprint-matching" may identify gram-negative and gram-positive bacteria, aerobes, anaerobes, mycobacteria, Nocardia spp., yeasts, filamentous fungus, and viruses, just like nucleotide-based techniques. Additionally, if the sample volume is sufficient, the turnaround time from sample acquisition. Compared to the days needed for cultures, it can take just a few minutes to an hour to get results.

In the clinical laboratory context, MALDI-TOF MS is being employed in the US as a confirmatory test to identify organisms that have already grown from culture. MALDI-TOF MS has previously been used to directly analyse urine and spinal fluid samples for pathogen identification from cerebrospinal fluid (CSF) and urine from cases of meningitis and urinary tract infections, respectively, without prior culture. Previous studies have examined the use of MALDI-TOF MS on infected human samples. The considerably higher yield available from urine and CSF samples make them especially suitable for direct analysis because MALDI-TOF MS needs between 103 and 104 cells per analyte for positive identification. Compared MALDI-TOF MS has been demonstrated to have excellent promise as an analytical technique for the characterization of various types of microbes, and has a gain in time in days compared to previous identification methods.

The breadth of organisms established in the database of the particular biotyper software that is used and the yield of pathogens in an analyte limit the reach of pathogen detection by MALDI-TOF MS similarly to other approaches outlined. In addition, polymicrobial endophthalmitis is quite uncommon, therefore this limitation may not be of much importance. However, MALDI-TOF MS is not suitable for the investigation of polymicrobial diseases. A significant barrier for some communities and health systems would be the availability of the equipment and software. MALDI-TOF MS should also be used to look at a larger range of organisms, such as additional bacteria, mycobacteria, fungus, and polymicrobial illnesses. The study of human endophthalmitis samples and well-designed in vivo animal models are required to verify the clinical utility of MALDI-TOF. For direct analysis without prior culture, use MS and automated AST. The variety of organisms listed in the database of the particular biotyper software in use restricts the range of pathogen identification. Additionally, sufficient numbers of bacteria need to be present in intraocular samples taken from patients for the methods we describe to be clinically applicable.

Furthermore, the precise impact of antimicrobials in endophthalmitis samples for MALDI-TOF MS analysis, such as the potential modifications to the microbial epitopes and molecular byproducts that may influence the peptide profile upon analysis, has yet to be fully explored. Unlike conventional microbiological methods, which only require the presence of particles of the offending organism for identification, MALDI-TOF MS requires the organism to be intact or alive for proper identification.

Discussion

However, quick and precise diagnostic methods are essential for endophthalmitis, and current diagnostic techniques have many drawbacks. The ability to identify microorganisms without prior culture could represent a novel and innovative shift in clinical microbiological methodology, even though more research and optimization models for the direct analysis of human ocular samples from cases of endophthalmitis with MALDI-TOF MS are required. The fact that MALDI-TOF MS can recognise finicky and relatively uncommon pathogens suggests that there may be room for improvement in the gold standard of pathogen detection in terms of speed, accuracy, and resource usage. In an era where the usefulness of targeted molecular medicines will help to safeguard against rising antibiotic resistance and its concomitant consequences, this is unquestionably of high therapeutic utility and significance morbidity and healthcare expenses [5-10].

Conclusion

In clinical microbiology, pathogen identification in infectious endophthalmitis presents significant problems. The biopsy volumes provided by the intraocular compartments are frequently insufficient, and the routine use of intravitreal antibiotics can stop the subsequent isolation of living organisms. Importantly, early infection detection is essential for the best possible management because of the rapid illness progression and threat to vision. A part of the recent developments in microbiology are covered in the studies on PCR, HTS, whole genome sequencing, and MALDI-TOF MS that were mentioned above. Each method has advantages and disadvantages, but they all share the desire to obtain the most clinical information with the least amount of resources. We may eventually be able to use the most effective aspects of these cutting-edge molecular approaches to create an improved technique that requires few processing steps and readily available analytical technology, and that can quickly offer reliable detection of a wide range of pathogens from tiny sample volumes, and resist the effects of antimicrobials or immune-mediated changes.

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Conflict of Interest

None.

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