

## Use of Broad-Range 16S rRNA Gene PCR Assay for Diagnosis of Bacterial Endophthalmitis Infections

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### Abstract

Endophthalmitis is a potentially sight-threatening condition and varies geographically in incidence and in cause. Early laboratory diagnosis and appropriate treatment prevents blindness in many patients of endophthalmitis. Currently bacterial infections in majority of patients are treatable. Prompt detection of infecting bacteria and appropriate antibiotic therapy, helps in saving lives and prevent debilitating complications. Identification of bacterial species in culture is still the mainstay in laboratory diagnosis, however in many cases, organisms cannot be detected and identified by standard culture method. Even after advances in automated culture detection methods the pathogen identification rate has not improved greatly. In almost half the patient samples, it fails to detect the causative agent. Introduction of PCR assay though had improved diagnosis, it requires multiple specific primers which is expensive and time consuming. In such cases, (where standard microbiological cultures fail), 16S ribosomal rRNA broad based PCR assay has the potential to be used for identifying the bacterial species. These techniques are rapid, reliable and bypass the need of sample culture. In the proposed work, we want to use broad-range PCR assay to specifically detect and identify bacterial species in infections like endophthalmitis that are negative by routine bacterial cultures. This study will also help in ascertaining the feasibility of these tests for etiological agent detection in such infections and could be a promising diagnostic tool in management of endophthalmitis.

**Keywords:** Endophthalmitis; 16S Ribosomal rRNA; Broad-Range 16S rRNA Gene PCR Assay; Exogenous Endophthalmitis; Endogenous Endophthalmitis

### Introduction

Endophthalmitis is one of the most dreaded sight-threatening intraocular infection often leading to avertable blindness varies geographically in incidence and in cause. Early laboratory diagnosis and appropriate treatment prevents blindness in many patients of endophthalmitis. Endophthalmitis is an ophthalmic crisis that can result in overwhelming ocular and systemic complications. The most common means of entry of infective microorganisms is through an external wound, such as trauma, surgery, or infected cornea. Such cases of endophthalmitis are termed as exogenous endophthalmitis. Endogenous endophthalmitis (EE), in contrast, results from the hematogenous spread of microorganisms from distant foci [1-3]. The first case of bacterial EE has been published in 1856 [5] and then consequently, a major review including approximately 335 cases of bacterial EE was published in 2003 [4], and the authors have recently updated their initial data by accommodating further reports [6]. Depending upon the severity of the disease, both medical and surgical interventions may be employed. Incidence of localized infections like endophthalmitis varies from 0.05% to 0.09% in India. Although prompt diagnosis and appropriate therapy can prevent blindness, laboratory diagnosis of endophthalmitis using conventional cultures suffers from low sensitivity and delay in reporting, leading to unfavorable patient outcomes.

Conventional culture is the first line laboratory diagnostic method to detect microorganisms from patient specimens, however they rely on the detection of viable organisms, requires samples to be processed immediately and are time consuming. To overcome such

problems and to increase the sensitivity and specificity, automated culture methods are being increasingly used in recent years. Although both conventional and automated culture methods are used in some tertiary care hospitals and established laboratories, still these have some limitations i.e. some uncultivable bacteria remains unrecognized. Such culture negative situations highlight the need for a rapid, broad-spectrum diagnostic assay that are able detect the causative organism and identify the emerging pathogens in situations where conventional culture and automated culture techniques have failed [7-10].

This review discusses the epidemiology, etiology and clinical diagnosis of endophthalmitis and identification of bacterial pathogens which were uncultivable with both conventional and automated BACTEC culture using broad range 16S rRNA gene PCR assay.

### Causative agents

The organisms responsible for endophthalmitis differ depending on the geographic location. In the developed world, gram positive organisms (*Streptococci*, *Staphylococci* and *Pseudomonas*) lead to the infection endophthalmitis, whereas gram negative organisms are more common and rarely fungi in the Asian population [11,12]. Asian studies have reported fungi as the causative organisms in approximately 11.1 to 17.54% of total cases of endophthalmitis, with the rest cases being accredited to bacterial causes [3,12].

### Clinical aspects

Initial symptoms are blurred vision and a red or aching eye that affects most of the patients. Pain and discomfort in eyes, thought to be a major indicative sign, can be absent in up to 25% of the cases [14]. Poor visual acuity, decreased media clarity, and poor fundus visualization are the main clinical signs. Hypopyon is the usual diagnostic sign of endophthalmitis that represents a layer of WBC in anterior chamber of eye, yet is unpredictable as a solitary indicator. General symptoms such as fever are present in endogenous endophthalmitis cases but often absent in exogenous endophthalmitis cases [15]. Bacterial endophthalmitis typically presents acutely, often within days of cataract surgery.

### Classification of endophthalmitis

Endophthalmitis is an infection that may be broadly categorized by clinical course (acute occurs 2 - 4 days postoperatively, and is most frequently due to *Streptococci*, *Staphylococcus aureus*, or Gram negative organisms versus chronic that can occur very early as 1 month postoperatively. *Propionibacterium acnes*, *Staphylococcus epidermidis*), by etiology (infectious versus non-infectious), by the route of entry of the causative agent to the globe (exogenous versus endogenous) and by the organism(s) involved (bacteria, fungi, parasites and rarely, viruses) [16]. Certain organisms tend to be associated with particular clinical settings, means of intraocular access and types of inflammation (acute, chronic non-granulomatous, chronic granulomatous or mixed cellular response). Infectious agents generally enters to the posterior segment of the eye following one of three routes: (i) following intraocular surgery (postoperative), (ii) following a penetrating injury of the globe (posttraumatic), or (iii) from hematogenous spread of bacteria to the eye from a distant anatomical site (endogenous). Although uncommon, endophthalmitis can also result from keratitis, an infection of the cornea which, if left untreated, can result in corneal perforation and intraocular seeding of organisms [16].

### Exogenous endophthalmitis:

Exogenous endophthalmitis refers to infections resulting from infringe of the globe exterior through surgery or trauma, or by fulminant progression of inflammatory processes such as keratitis or scleritis. Most postoperative endophthalmitis develops after cataract surgery as most of these procedures are performed annually worldwide. Both acute endophthalmitis and chronic forms may occur. Other procedures associated with varying risks of endophthalmitis include corneal surgeries (penetrating keratoplasty, keratoprosthesis insertion and refractive corneal surgeries), vitreous procedures (intravitreal injections, vitrectomies), glaucoma surgical treatments (blebs, glaucoma valve placements), procedures to correct retinal detachment including scleral buckling, and other miscellaneous ocular surgeries, even strabismus correction. Most cases are sporadic but occasionally are clustered, suggesting contaminated materials/solutions or problems with instrument sterilization likely are responsible [17,18]. In these situations, unusual bacterial pathogens may be found.

### Postoperative endophthalmitis

Most postoperative endophthalmitis develops after cataract surgery as millions of these procedures are performed annually worldwide. In general, procedures including phacoemulsification and intraocular lens implantation with a higher risk for acute postoperative endophthalmitis (secondary intraocular lens implantation and penetrating keratoplasty [corneal transplantation]) are those with a greater potential for wound leaks with subsequent intraocular bacterial contamination. Generally studies have found coagulase negative *Staphylococcus* is the top pathogen [19-21,23] comprising in some series more than 60% of isolates. *Enterococci* and *streptococci* were also frequent in a study from Sweden. However, in 2 reports from India, the incidence of Gram positive and Gram negative bacteria was approximately equal and more cases of *Pseudomonas aeruginosa* endophthalmitis were identified [22,23]. Although less common than bacterial cases, fungal endophthalmitis can occur post cataract surgery [24,25] and is more frequent in Indian series where detection of *Aspergillus* sp. eclipses *Candida* spp [25].

Postoperative endophthalmitis may also occur weeks to years following surgery which is likely due to either seizure of low-virulence organisms introduced at the time of surgery or to delayed inoculation of organisms. In the former case, *Propionibacterium acnes* is the most common microorganism encountered, and clinically evident low-to moderate-grade inflammation may occur weeks to months after surgery.

### Posttraumatic Endophthalmitis

Postoperative endophthalmitis has been reported following nearly every type of ocular surgery occurs most frequently following cataract surgery. Posttraumatic endophthalmitis are of two types: acute and chronic. Most cases of acute post traumatic endophthalmitis present within 1 - 2 weeks, usually 3 - 5 days after the surgery. Initial symptoms included are rapidly progressive, including pain, red eye, ocular discharge, and blurring with signs decreased visual acuity, lid swelling, conjunctival and corneal edema, anterior chamber, hypopyon, vitreous inflammation, retinitis, and blunting of red reflex. On the other hand chronic endophthalmitis which manifest usually after several weeks or months of surgery [26-28].

### Endogenous endophthalmitis

Endogenous endophthalmitis results from the introduction of organisms into the posterior segment of the eye as a result of hematogenous spread of infection. Endogenous bacterial endophthalmitis is much less frequent than exogenous bacterial endophthalmitis with an incidence of 2 - 11% [29-32]. Populations at greatest risk include immunocompromised patients or those on immunosuppressive therapy, patients with prolonged indwelling devices, and intravenous drug abusers [33,34]. Common causes of endogenous bacterial endophthalmitis include *S. aureus*, *B. cereus*, and gram-negative organisms, including *Escherichia coli*, *Neisseria meningitidis*, and *Klebsiella* spp. [35-38]. Patients often complain of blurred vision with ocular pain and most but not all have evidence of systemic illness. Examination reveals hypopyon and hazy ocular media [38,39]. Both Gram positive and Gram negative bacteria have been reported as pathogens with incidences differing around the world. For example, several reports of East Asian populations [40-43] have clearly documented *K. pneumoniae* as the predominant endogenous endophthalmitis cause, with hepatobiliary infections (especially liver abscesses), as the initial disease site and diabetes mellitus a common co-existing condition [41]. Elsewhere in the world, *S. aureus*, Group B *Streptococcus* and *S. pneumoniae* have been more frequent [44-46].

### Laboratory diagnosis

Endophthalmitis is a clinical judgment supported by culture of the vitreous. Negative cultures vitreous specimens do not prohibit the diagnosis, since 20% - 30% of endophthalmitis cases are culture negative. Diagnosis of vitreous for Gram stain is commonly engaged for identification; although helpful when positive for microbes, still not very sensitive. Earlier, culture has been the only available means of determining organism(s) responsible for endophthalmitis. The disadvantage of these techniques is that depending on the type of infections 40 - 90% of microorganisms cannot be cultivated under laboratory conditions [22]. Molecular diagnostic techniques have confirmed a pathogen in many culture-negative cases; as a result these techniques may play a significant role in diagnosing endophthalmitis in the future. Recently, PCR has been found to be an important diagnostic technique and has been reported to greatly aid in bacterial detection

[48]. After wide spread use of PCR assay in disease diagnosis in early 1990s these have been widely used for microbial detection, most commonly bacteria from clinical specimen in infections particularly in bacterial infections. These have proven to be highly sensitive and specific. Initially the PCR assays were conducted for particular suspected bacterial pathogen using specific primers, the assays were highly sensitive and specific [22,47]. This was cumbersome and expensive [48]. Thereafter in last decade broad range PCR assay was developed which uses primers targeted at conserved regions of bacterial ribosomal genes. This method has the potential to detect bacterial DNA from any bacterial species [49] and is particularly valuable for patients under antibiotic therapy.

### **Broad range PCR assay**

Broad range PCR assay is useful in accurate determination of regional agent-specific infectious diseases can provide critical information for local authorities to optimize public health prevention strategies and maximize the use of limited available resources, particularly in developing countries. In recent years, advances in genomics have opened new avenues for pathogen detection targeting conserved regions of bacterial ribosomal genes. This method has the potential to detect bacterial DNA from any bacterial species [53]. Assays like broad range PCR assay has potential to detect causative agents from many more culture negative infections. Though automated culture detects significantly more number of bacterial infections than the conventional culture, broad-range PCR assay could detect far more infections. Therefore, broad-range PCR assay could be a promising diagnostic tool in management of endophthalmitis. In this study we intend to focus on determination of exact bacterial etiology in localized infections like endophthalmitis, particularly in culture negative ones using molecular methods like broad-range PCR assay.

### **Treatment and prevention**

Intravitreal drug administered in all cases of bacterial endophthalmitis are vancomycin and ceftazidime/amikacin. Systemic antibiotics which have been considered as good intravitreal are fluoroquinolones. On the other hand topical/subconjunctival/oral corticosteroids have not yet been proven useful and valuable. Topical steroids considered beneficial in case of mild/severe inflammation during endophthalmitis. Early initiation of treatment (within hours) is critical to prevent blindness.

### **Conclusions**

Treatment of endophthalmitis till date remains a challenge. Early detection, together with appropriate and timely treatment, may reduce vision loss associated with endophthalmitis. Though conventional culture methods considered as gold standard for microbial detection, still have limited scope in terms of sensitivity and specificity. Automated BACTEC culture could detect more number of bacterial pathogens than conventional culture still some of the bacterial pathogens remains unidentified. The limitations of conventional culture and automated BACTEC culture advocated the development of molecular techniques. The nucleic acid amplification technology has opened a new vista for uncultivable microbe detection and identification. At present, broad range PCR assay have become more important in clinical diagnostic laboratory settings because of its rapidity, sensitivity and specificity. It has a tremendous advantage over cultures, especially for some slow-growing bacteria such as mycobacteria and in detecting some culture negative bacteria responsible for infection. Hence, it may be suggested, to use broad range PCR assay in laboratory diagnosis in endophthalmitis patients, which will be helpful in guiding for a correct therapy and management of the infections.

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### **Conflict of Interests**

All the authors declare that there are no conflicts of interest related to this review article.

### **Informed Consent**

Consent was obtained from all individual participants included in the presentation of review article.

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