Identification of *Curvularia lunata* by polymerase chain reaction in a case of fungal endophthalmitis

Deepu Alex, Dongmei Li, Richard Calderone, Stephen M. Peters

**Abstract**

*Curvularia* is a dematiaceous mold that infects plant species and is found in the soil. In humans, it is known to cause keratitis after trauma to the eye. We report the findings of persistent fungal endophthalmitis in a 74-year-old female patient who had undergone prior cataract surgery. Mold identification and antifungal susceptibilities were done on 2 separate samples of vitreous fluid and they were found to be consistent with *Curvularia lunata* by the use of PCR amplification methods.

1. Introduction

*Curvularia* is a hyphomycete found in plants and soil of tropical and temperate countries. It is associated with open globe injuries and is a common cause of secondary fungal keratitis after diagnostic aspiration [1,2]. To date, however, only five known reports of endophthalmitis caused by *Curvularia spp.* have been documented [2–6].

A case of persistent fungal endophthalmitis was diagnosed in a 74-year-old female patient who had previously undergone cataract surgery. Initial identification of a *Curvularia* species was done in our laboratory based on morphology. The identification was then confirmed by the PCR amplification of the internal transcribed spacer (ITS) region specific to *Curvularia lunatus* and subsequent sequencing of the PCR amplification product. Antifungal susceptibility testing was also performed against a range of antifungal agents and the results were used as a guide in treatment.

2. Case

A 74-year-old woman had cataract surgery with radial keratotomy performed on her left eye in August 2011. She was apparently well with 20/25 vision in her left eye following surgery. In November 2011, she noticed sudden decreased vision in her left eye and presented to the emergency room with this complaint. She was found to have a trace hypopyon and a whitish material or plaque involving areas behind the lens. She was thought to have bacterial endophthalmitis and was prescribed antibiotic drops before discharge. The next morning, she returned reporting worsening of vision in her left eye. She was taken to surgery where a left eye pars plana vitrectomy was performed and intraocular vancomycin and ceftazidime were injected to counteract the infective process. The vitreous samples taken at this time did not show any growth of microorganisms up to 21 days.

The patient did well postoperatively with a steady improvement in vitreous clarity. The inflammation in the anterior chamber decreased and her vision, although limited, was stable. In December 2011, a new 1-mm hypopyon with increased opacification of the vitreous was seen. She was then taken to emergency surgery where a left eye vitrectomy, aspirate of vitreous fluid and lysis of iridocorneal adhesions were done. At this time, intravitreal injections of vancomycin, amikacin and triamcinolone were also administered. The biopsy was inconclusive and the vitreous cultures were negative for up to 21 days.

In January 2012, she began to complain of cloudiness in vision and on examination was found to have a new vitreous opacity. The patient was once again taken to surgery where a vitrectomy was performed and cultures were taken from the vitreous fluid. In 6 days, the microbiology laboratory identified mold species from 2 separate cultures. Based on morphology and PCR studies, the organism was identified as *Curvularia spp.* Vitreous cultures were repeated 2 weeks later and the same organism was
identified. Antifungal susceptibility testing was performed against a range of antifungal agents and the results are shown in Table 1. There are currently no CLSI interpretative criteria or guidelines for mold susceptibility by microbroth dilution. Thus, the MIC results were reported as μg/mL without any interpretations. In the absence of clinical endpoints for mold infections, the average in-vitro antifungal activity of specific agents like voriconazole was used as a reference to determine susceptibility. For example, Sabatelli et al. reported that the average MIC₉₀ of voriconazole against all molds is 2.0 μg/ml [7]. In this context, the strain of Curvularia was most likely to be susceptible to voriconazole treatment (MIC = 0.2 μg/ml). The patient was administered intraocular injections of amphotericin B and voriconazole twice after vitrectomy. She was also started on oral voriconazole therapy which she continued to take for the next 4 months (January–April). Repeat cultures of vitreous fluid over the next 2 months failed to grow any organisms.

In spite of this, the patient had gradual worsening of vision in the left eye over the next 3 months with evidence of retinal detachment. In view of this chronic course with minimal improvement, left eye enucleation was performed in April 2012. Histopathology studies showed areas of chronic hemorrhagic retinal detachment. The vitreous cavity was filled with blood. The retinal layers showed a disorganized architecture which was consistent with the chronic nature of the detachment. Special stains for microorganisms were negative.

3. Discussion

Three ubiquitous species of Curvularia have been recovered from human infections. They are Curvularia lunata, Curvularia pallescens and Curvularia geniculata. Of these, Curvularia lunata is more commonly found in immunocompromised individuals. They are spread via inhalational or dermal inoculation routes. Thus, they are seen after corneal perforation or surgery, presence of peritoneal and venous catheters and in IV drug abusers [8]. A fatal case of cerebral phaeohyphomycosis due to C. lunata was reported in an immunocompetent patient with no prior history of respiratory or sinus tract infections [9]. Other reported infections with Curvularia spp. include a brain abscess in a patient with

| Antifungal agent | MIC (in μg/ml) |
|------------------|----------------|
| Voriconazole     | 0.12           |
| Ketoconazole     | 0.12           |
| Fluconazole      | 4.0            |
| Amphotericin B   | 0.12           |
| 5-Fluorouracil   | ≥ 128          |
| Itraconazole     | 0.03           |

Table 1: MIC values of 6 antifungal agents against the strain of Curvularia lunata by the CLSI broth microdilution method.

![Fig. 1](image-url). (Top) appearance of Curvularia lunata cultures on Potato Dextrose Agar (A) and reverse of petri dish (B) after 7 days of incubation at 30°C. (Bottom) microscopic typical appearance of multiple-cells conidia (C) and sympodial germination (D) after 5 days of incubation at 30°C on water agar, shown by phase-contrast microscopy. Magnification x20.
chronic sinusitis [10] and peritonitis in an elderly patient undergoing peritoneal dialysis [11]. The differential diagnosis of *Curvularia lunata* includes *Bipolaris* spp., and *Exserohilum* spp. [12]. All of the above mentioned fungi are common saprophytes on plant material and in soil, developing dark colonies (dematiaceae) and pigmented multicelled spores. However, the spores are not routinely found on direct examination of smears or culture samples and the critical tests needed to accurately identify these genera are lacking. The differentiation relies upon the direct examination of sporulating structures under microscopy. The key feature of *Curvularia sp.*, is the production of true septate conidia (cross-wall) while the conidial cells in *Bipolaris* and *Exserohilum* are compartmentalized by distosepta which means that they are contained in sacs whose surfaces do end on the outer wall of the conidium.

The primary culture of the patient's vitreous specimens was a black mold [first cultured on Sabouraud Dextrose Agar (SDA, Oxoid) from vitreous specimens at 30 °C for 72 h]. Upon subculture to PDA agar media (Potato-Dextrose-Agar) the colonies were initially gray but changed to grayish-black as the colony aged. The bottom of each colony (Fig. 1B) was black without diffusion of pigment into the medium. The top of the colony was velvety to floccose showing moderate growth (55 mm in 7 days) at 30 °C (Fig. 1A). Microscopic examination of growth revealed both hyaline and pigmented septate hyphae in which conidiophores arose terminally or laterally. The brownish conidia were broadly ellipsoidal or clavate, smooth-walled and for the most part contained 4 septa (Fig. 1C). The conidia were variable in size (21.0–31.0 μm by 8.5–12.0 μm) and were produced in sympodial order to leave dark brown scars on conidiophores (Fig. 1D). The sub-terminal cells of the conidia were curved, larger and darker. The appearance of this clinical isolate at both macroscopic and microscopic levels showed dematiaceous fungi with multicelled conidia and a sym- podial germination pattern that is consistent with *Curvularia* [13]. A cytology specimen of the vitreous fluid also showed septations and branching consistent with the morphology of *Curvularia* species (Fig. 2).

The difficulty in identifying *Curvularia sp.* on a morphological basis alone poses a diagnostic dilemma. Not infrequently, the morphologic patterns may not be clear due to factors like absence of spores, prior antifungal treatment and slow growth of the organism. In such circumstances, molecular methods can be of great assistance. For most Basidiomycetes and some Ascomycetous fungi- including *Curvularia lunata* (*Cochliobolus lunatus*), rDNA exists as repeat units composed of major transcripts (18S, 5.8S and 28S) and an internal transcribed spacer (ITS) region. Because of the higher degree of variation of this ITS region, it has been widely used in molecular taxonomy to differentiate at species levels or sometimes even within species [14–16]. The primers ITS1 and ITS2 were as follows. ITS1 5′-TCC GTA GGT GAA CCT GCG G-3′ and ITS2 5′-GCT GGG TTC TTC ATC GAT GC-3′. The PCR amplification was performed using standard methods [17]. The product of the amplification was 270 bp which was sequenced with GeneWiz, Inc. The sequencing data aligned with C. lunatus rDNA in NCBI gene bank (DQ337381.1) and resulted in a 100% identity match. Thus, the isolate was confirmed as *Curvularia lunata* on the basis of this molecular test, with the ITS sequence exactly complementing the rDNA sequence of this species.

*Curvularia* spp. is increasingly being identified as a cause of infections in immunocompromised individuals. In this report, we have identified *C. lunata* as a cause of fungal endophthalmitis in an elderly woman. Therapy with intravenous voriconazole and amphotericin B helped clear the vitreous fluid of organisms as seen from subsequent negative cultures. However, the clinical deterioration of the patient in spite of constant monitoring and therapy is significant. Since these infections are more prevalent in populations with multiple co-morbidities (as is seen in immunocompromised patients), there are multiple factors that determine if the patient will go into remission for chronic mold infections. The absence of clear in-vitro clinical end-points to treat these organisms adds to the complexity of this disease. Based on the identification of this organism as a cause of vitreous infection, it would be prudent to consider as a differential diagnosis in cases of endophthalmitis that are resistant to conventional antibacterial therapy.

**Conflict of interest**

There are none.

**Acknowledgments**

We would like to thank Dr. Pedro de Brito, Department of Pathology and Laboratory Medicine at Medstar-Georgetown University Hospital for the images of the vitreous fluid cytology specimens.

**References**

[1] Pathengay A, Miller DM, Flynn Jr. HW, Dubovy SR. *Curvularia* endophthalmitis following open globe injuries. *Archives of Ophthalmology* 2012;130(5):652–4 (May 1).

[2] Kaushik S, Ram J, Chakrabarty A, Dogra MR, Brar GS, Gupta A. *Curvularia lunata* endophthalmitis with secondary keratitis. *American Journal of Ophthalmology* 2001;131(1):140–2.

[3] Bhala S, Narang S, Sood S, Mithal C, Arya SK, Gupta V. Microbial contamination in open globe injury. *Nepalese Journal of Ophthalmology* 2012(7):84–9 (Jan 4).
[4] Berbel RF, Casella AM, de Freitas D, Höfling-Lima AL. Curvularia lunata endophthalmitis. Journal of Ocular Pharmacology Therapy 2011;27(5):535–7 (Oct).

[5] Ehlers JP, Chavala SH, Woodward JA, Postel EA. Delayed recalcitrant fungal endophthalmitis secondary to Curvularia. Canadian Journal of Ophthalmology 2011;46(2):199–200 (Apr).

[6] Pathengay A, Shah GY, Das T, Sharma S. Curvularia lunata endophthalmitis presenting with a posterior capsular plaque. Indian Journal of Ophthalmology 2006;54(1):65–6 (Mar).

[7] Sabatelli F, Patel R, Mann PA, Mendrick CA, Norris CC, Hare R, et al. In vitro activities of posaconazole, fluconazole, itraconazole, voriconazole, and amphotericin B against a large collection of clinically important molds and yeasts. Antimicrobial Agents and Chemotherapy 2006;50(6):2009–15 (Jun).

[8] Mold and Bacteria Consulting Laboratories. Available from: [http://www.moldbacteria.com/tag/curvularia] [accessed 25.07.13].

[9] Carter E, Boudreaux C. Fatal cerebral phaeohyphomycosis due to Curvularia lunata in an immunocompetent patient. Journal of Clinical Microbiology 2004;42(11):5419–23 (Nov).

[10] Gadgil N, Kupferman M, Smitherman S, Fuller GN, Rao G. Curvularia brain abscess. Journal of Clinical Neuroscience 2013;54(1):173–5 (Jan).

[11] Pimentel JD, Mahadevan K, Woodguy A, Sigler L, Gibas C, Harris OC, et al. Peritonitis due to Curvularia inaequalis in an elderly patient undergoing peritoneal dialysis and a review of six cases of peritonitis associated with other Curvularia spp. Journal of Clinical Microbiology 2005;43(8):4288–92 (Aug).

[12] Revankar SG, Sutton DA. Melanized fungi in human disease. Clinical Microbiology Reviews 2010;23(4):884–928 (Oct).

[13] Larone DH. Medically important fungi: a guide to identification, 147. Washington DC: ASM Press; 1995.

[14] Hofstetter V, Clemencin H, Vilgalys R, Moncalvo JM. Phylogenetic analyses of the Lyophylleae (Agaricales, Basidiomycota) based on nuclear and mitochondrial rDNA sequences. Mycological Research 2005;109:1043–59.

[15] Zhihong W, Yoshihiko T, Goran B, Xiao-Ru W. 18S rRNA gene variation among common airborne fungi and development of specific oligonucleotide probes for detection of fungal isolates. Applied and Environmental Microbiology 1999;65(9):3899–97.

[16] Borneman J. PCR primers that amplify fungal rRNA genes from environmental samples. Applied and Environmental Microbiology 2000;66(10):4356–60 (Oct).

[17] Martin KJ, Rygiewicz PT. Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. BMC Microbiology 2005;66(10):1–11 (May).