Colletotrichum truncatum species complex: Treatment considerations and review of the literature for an unusual pathogen causing fungal keratitis and endophthalmitis

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A B S T R A C T

We present a case of Colletotrichum truncatum species complex fungal keratitis and endophthalmitis in an 87-year-old immunocompetent male in whom oral triazole antifungals were contraindicated. The patient had recently returned from 4 months in Jamaica with a one month history of progressively increasing pain and inflammation in his left eye. Corneal samples grew a filamentous fungus and internal transcribed spacer sequencing polymerase chain reaction confirmed the presence of C. truncatum species complex. Samples showed no microbial growth.

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1. Introduction

Fungal endophthalmitis is a vision-threatening infection that is usually seen in immunocompromised individuals with fungemia, intravenous drug users, or immunocompetent individuals following direct inoculation from penetrating ocular trauma [1,2]. Fungal endophthalmitis is generally associated with poor visual outcomes and retinal detachment is a frequent occurrence [2]. However, fungi account for only 2–10% of all endophthalmitis cases making this serious condition quite rare [1].

Yeasts, especially Candida albicans, are the most common cause of culture-proven fungal endophthalmitis, followed by molds, usually Aspergillus species [2,3]. A limited number of species of Colletotrichum have been reported to cause infection in humans. The majority of these cases have been keratitis with a few endophthalmitis cases due to Colletotrichum dematium [4] and Colletotrichum truncatum [5].

The treatment of fungal endophthalmitis is a serious challenge for ophthalmologists as the outcome is unfavorable in a considerable number of cases. The treatment protocol in fungal endophthalmitis is still not optimized due to the low incidence of this disease at most centers. Various regimens combining oral and topical antifungals have been reported, including triazole and polyene antifungals. Unfortunately triazoles have been responsible for a number of clinically significant drug interactions. Triazoles are inhibitors of lanosterol 14 α-demethylase, which prevents lanosterol production and thereby cell membrane integrity. They also inhibit other cytochrome P450 enzymes, including CYP3A4 and CYP2C9. Therefore a number of CYP3A4 and CYP2C9 substrate drugs, such as alpha-adrenergic antagonists (ex. tamsulosin) are contraindicated, especially when administered in the high doses needed to treat fungal endophthalmitis [6,7].

This case report describes a patient on tamsulosin with C. truncatum fungal keratitis and endophthalmitis. Susceptibility data for this species is presented and we review the literature on the treatment and outcomes after ocular infections from Colletotrichum species.
1.1. Case

An 87 year-old man who recently returned to Canada after spending 4 months in Jamaica, presented (day 0) with a 1 month history of progressively increasing pain, redness, excessive tearing, decreased vision, and lid swelling in his left eye. He denied any history of ocular trauma or contact lens wear. The patient had decreased hearing but was otherwise healthy and was taking acetylsalicylic acid 81 mg daily. He reported that his doctor in Jamaica prescribed him an unknown topical ophthalmic solution. Once back in Canada he was seen by an optometrist who treated him with topical moxifloxacin and referred the patient 3 weeks later given the patient's worsening condition.

On examination of the patient's left eye, uncorrected visual acuity was light perception. The left pupil was fixed and mid-dilated. Intraocular pressure (IOP) was 22 mmHg. Slit lamp examination revealed limbal neovascularization; conjunctival injection; inferior keratic precipitates; a 4 x 5 mm², 90% thinned area of corneal stromal haze with no overlying epithelial defect; a dense cataract; and a shallow anterior chamber with temporal iridocorneal touch. Dilated fundoscopy was difficult but the retina appeared flat. A provisional diagnosis of herpes simplex immune stromal keratitis with uveitis was made. The patient was started on oral acyclovir 400 mg 5 times daily, as well as topical prednisolone acetate 1% four times daily, timolol maleate 0.5% twice daily, artificial tears four times daily, and Lacrilube ointment (Al-lergan, Irvine, CA) before bed.

Two weeks later (day 15), the patient's pain was improved with stable visual acuity, stable IOP, and diminished conjunctival injection. At the 3 week follow-up appointment (day 22), the cornea appeared hazier and had developed an ectatic bulge. A hypopyon formed 360 degrees, Descemet's membrane folds consistent with early post-operative stromal edema and pigmented precipitates on the endothelium were present. On POD 4 (day 55), the fungal culture became positive for a filamentous fungus, which grew on Sabouraud's agar with gentamicin, brain heart infusion agar with chloramphenicol, cycloheximide and gentamicin, and on Inhibitory Mold Agar. The initial colonial appearance was of a flat white colony with grey speckles and a beige periphery, with a grey reverse (Fig. 1). The culture was referred to the reference mycology laboratory, where it was found to be non-sporulating when examined microscopically. It was sent for ITS2 (internal transcribed spacer) sequencing PCR for identification. By POD 6 (day 57), the preliminary pathology report documented the presence of hyphae in the corneal tissue (Fig. 2). The molecular identification was reported as Colletotrichum capsici. After 7 days incubation, identifying features of sporulation were observed on microscopy of the colony, including dark brown, spherical and setose conidia, brown, rigid, smooth-walled setae, brown variably shaped appendages, and one-celled falcate conidia (Fig. 3) with an acute apex.

Further molecular testing, based on DNA sequencing using a combination of different primer sets and the MycoBank (CBS-KNAW) reference database indicated that the fungal identification was most consistent with C. truncatum species complex. Loci assessed included: D1/D2 (100% C. truncatum, accession No. DQ286159), ITS3/ITS4 (100% C. truncatum, accession No. AJ301944), beta-tubulin (C. truncatum 99.798%, accession No. HM575221.1), and translation elongation factor 1α (C. truncatum 96.538). Some entries indicated a high degree of homology with C. capsici, which is a synonym of C. truncatum and also of Colletotrichum jasminigenum, which is part of the C. truncatum species complex.

Since the patient was receiving tamsulosin for his advanced BPH, an oral triazole was contraindicated. Infectious diseases was consulted and recommended that 0.15% amphotericin B could be used. Intravitreal injection of amphotericin B 0.01 mg/0.1 ml was attempted but the patient could not tolerate it. A subconjunctival depot was administered (day 78).

The patient once again was lost to follow-up and presented one month later (day 112) with a painful blind eye, mildly injected conjunctiva, clear PK graft with intact epithelium, a large fibrin plaque adherent to the endothelium, and dense vitritis on B-scan ultrasound, consistent with presumed fungal endophthalmitis. At this time, the patient requested and underwent left eye evisceration and reconstruction (day 129). GMS stained section from the evisceration specimen showed fungal elements in the corneal graft and in the anterior chamber (Fig. 4).
2. Discussion

Fungal endophthalmitis is an uncommon, but potentially blinding condition requiring early, aggressive therapy. Unfortunately, fungal endophthalmitis is often a diagnostic and treatment challenge for ophthalmologists because it can masquerade as other more common causes of keratitis or uveitis. Some studies have reported rates of misdiagnosis for *Candida endophthalmitis* approaching 50% [1]. Non-candida species have been infrequently documented. This report describes an even less common case of *C. truncatum* keratitis and endophthalmitis identified using molecular methods. The diagnosis was made from the pathological and microbiological analysis of the corneal tissue submitted after PK surgery 3 months after the onset of the patient’s symptoms.

*Colletotrichum* species are coelomycetous soil fungi that are common in tropical and subtropical regions of the world as saprophytes of plants. They usually enter tissue by trauma with organic matter [8]. Traumatic implantation is important for the initiation of infection, as the conidia (spores) are contained in the asexual fruiting body and are not freely released. A small case series from India reported a history of ocular trauma from 5 of 6 patients with ocular infection due to *C. truncatum* and the sixth patient was 70 years old so trivial trauma that went unnoticed could not be excluded [5]. Similarly, no obvious history of trauma could be elicited from our 87-year-old patient so it was possible that he suffered minor trauma that was disregarded due to HSV-related neurotrophic corneal disease. It is probable that our patient acquired the fungus in Jamaica, either through swimming or other contact with environmental fungi, aided by mild trauma or pre-existing disease.

*Colletotrichum* species may be slow to sporulate and may show subtle morphological features, which makes the conventional microscopic identification difficult. Thus, the molecular approach is very helpful in making a rapid genus and species diagnosis. Phylogenetic analysis based on the nucleic acid sequence of the ITS region of the organism’s ribosomal DNA is the most common method for fungal identification, but is unsatisfactory for determining the specific species of *Colletotrichum*, though it reliably identifies the *Colletotrichum* genus and major clades within it. It has been shown that increasing the number of loci that are
This is an important consideration since BPH and tamsulosin usage is common in elderly males. The ophthalmologist should be aware of these potential drug interactions when developing a treatment plan for a patient on an alpha-blocker who develops an ocular fungal infection and seek the expertise of infectious disease specialists. In our case, topical amphotericin B was the recommended agent.

Regarding specific treatments for *Colletotrichum* species, there is very little information on drug susceptibility in the literature. The clinical data indicate that ocular infections due to *Colletotrichum* species (*C. dematium, Colletotrichum gloeosporioides*) respond well to natamycin [11–15] although amphotericin B is the treatment of choice in vitro [12,14,16]. MIC results from two studies indicated that *Colletotrichum* species are susceptible to amphotericin, itraconazole, miconazole, micafungin, and voriconazole, but resistant to natamycin, 5-fluorocytosine, and fluconazole [12,15,16]. Unfortunately there are no interpretive guidelines and no in vitro-in vivo correlation studies for *Colletotrichum* species infections. Marangon and colleagues noted that even though voriconazole did very well in their in-vitro susceptibility analysis, it was not effective in clinical use for two of their patients with *Colletotrichum* keratitis [13]. In both of these cases, topical treatment with a 1% solution failed to control the infection, but resolved with topical natamycin [13]. Giaconi and colleagues reported similar results [14].

The results of our in-vitro antifungal susceptibility testing are the first to be published in the literature for *C. truncatum* (Table 1). Like *C. dematium* and *C. gloeosporioides*, *C. truncatum* is also susceptible to amphotericin B in addition to posaconazole. In contrast to the literature, we found that in addition to 5-fluorocytosine, *C. truncatum* showed high and probably resistant MICs to voriconazole and relatively high MICs to ketoconazole, itraconazole, and miconazole. Unfortunately, these data were not available during the course of the patient’s care to aid in the management of our patient.

Additional successful treatments specific to fungal endophthalmitis used in various combinations with natamycin include oral and topical voriconazole [12,16,17] as well as topical antibiotics [11,12], intravitreal amphotericin-B [5,10], systemic steroids [18], and vitrectomy [10]. Additional details regarding the treatment and outcomes of reported cases of *Colletotrichum* species ocular infections are summarized in Table 2.

In conclusion, although *Colletotrichum* species infections in humans are rare, this case report highlights the expanding spectrum of infectious agents responsible for fungal keratitis and endophthalmitis and the treatment considerations that may arise in a patient with a contraindication to anti-fungal triazoles. Any uncommon fungus should not be disregarded as a contaminant when isolated from important, especially intraoperative specimens in which the direct microscopic examination of the primary specimen is positive for fungal elements. In addition, it is

### Table 1

| Drug            | MIC (μg/mL) |
|-----------------|-------------|
| Amphotericin B  | 0.5         |
| 5-Fluorocytosine| > 64        |
| Itraconazole    | 2           |
| Ketoconazole    | 2           |
| Posaconazole    | 0.5         |
| Voriconazole    | 16          |
| Miconazole      | 2           |

Fig. 5. PAS-stained slide shows septated hyphae X20.

sequenced, increases the reliability of distinguishing individual species in this genus [9]. Thus, when we sequenced 3 other loci (especially beta-tubulin and D1/D2), it became apparent that this isolate fell into the *C. truncatum* species complex. *C. capsici* is an outmoded synonym for *C. truncatum*, which is still present in the GenBank sequence database (National Center of Biotechnology Information). In a small prospective case series of fungal ocular infections from India, Shivaprakash et al. misidentified all 6 strains causing ocular infection as *C. dematium* due to its close morphological resemblance to *C. truncatum* [5]. This evidence emphasizes the need for molecular techniques for species identification.

The final microbiology report stated that the isolated organism, *C. capsici*, was a “probable contaminant,” citing its known status as a plant pathogen. Since the original specimen sent for culture was very small, direct microscopy was not performed, which, if positive, would have helped to support the pathogenic role that this fungus was playing in this particular patient, as opposed to considering it a probable contaminant. However, light microscopy of the pathology tissue sample demonstrated the intracellular presence of the organism within the corneal stroma (Fig. 5), thus supporting its pathogenic role. The lab was initially unable to perform susceptibility testing for the isolate because of its poor ability to sporulate, however, by the time it was sent to a reference antifungal susceptibility testing laboratory (Fungus Testing Laboratory, University of Texas), it had sporulated to the extent that laboratory, University of Texas), it had sporulated to the extent that it was still present in the laboratory, University of Texas), it had sporulated to the extent that it was no longer able to sporulate, however, by the time it was sent to a reference laboratory, University of Texas), it had sporulated to the extent that it was not effective in clinical use for two of their patients with *Colletotrichum* keratitis [13]. In both of these cases, topical treatment with a 1% solution failed to control the infection, but resolved with topical natamycin [13]. Giaconi and colleagues reported similar results [14].
Table 2
Summary of reported treatment and outcomes of *Colletotrichum* species ocular infections.

| Reference         | Clinical specimen type | Species (number of cases) | Method of identification | Treatment | Duration of Treatment | Outcome                          |
|-------------------|------------------------|---------------------------|--------------------------|-----------|----------------------|----------------------------------|
| Fernandez et al.  | Corneal scrape          | *Colletotrichum* spp. (4) | Direct microscopy, positive culture | Topical natamycin; ± oral amphotericin B and itraconazole; or intravitreal amphotericin B, TPK, oral fluconazole | Natamycin: 26–54 days; amphotericin B: 27 days | Final Va = 20/30 – NLP           |
|                   |                        | *C. gloeosporioides* (3)  | Direct microscopy, positive culture |                                      | Natamycin: 25–30 days; amphotericin B: 90 days; itraconazole: 20 days | Final Va = 20/20–20/60           |
|                   |                        | *C. dematium* (3)         | Direct microscopy, positive culture | Topical natamycin                   | 50–52 days                        | Final Va = 20/20–20/25           |
| Kaliamurthy et al.| Corneal scrape          | *C. dematium* (1)         | Direct microscopy          | Topical natamycin and ciprofloxacin, hourly (6); Topical ciprofloxacin and tobramycin | 10–60 days                        | 2 needed TPK; Final Va = 20/20–20/400 |
| [11]              |                        |                           |                          | Topical voriconazole, hourly         | 18 days                           | Failed voriconazole; Resolved with 3 months of topical natamycin |
| Marangon et al.   | Corneal scrape          | *Colletotrichum* spp. (14) | –                        |                                      |                                   | Failed voriconazole; resolved with 3 months of topical natamycin; Final Va = 20/200 |
| [13]              |                        | *C. dematium* (1)         | Direct microscopy         | Topical voriconazole                 | 16 days                           |                                   |
| Giaconi et al.    | Vitreous tap            | *C. dematium* (1)         | Direct microscopy ± positive culture | PPV; intravitreal amphotericin B; intravitreal dexamethasone; oral fluconazole or itraconazole | 6 weeks                           | Final Va ≥ 20/400; attached retina, preserved globe anatomy, no active inflammation |
| [14]              |                        |                           |                          |                                      |                                   | Final Va = 20/25                   |
| Chakrabarti et al.| Vitreous tap            | *C. dematium* (1)         | Direct microscopy         | Topical voriconazole, topical natamycin, oral voriconazole | 3 months                          |                                   |
| [4]               |                        |                           |                          |                                      |                                   |                                   |
| Mitani et al.     | Corneal scraping        | *C. gloeosporioides* (1)  | Direct microscopy, positive culture | TOPICAL voriconazole, TOPICAL natamycin, ORAL voriconazole | Not available                     | Final Va = 20/25                   |
| [16]              |                        |                           |                          |                                      |                                   |                                   |
| Takezawa et al.   | Corneal scraping        | *C. gloeosporioides* (1)  | Direct microscopy, positive culture | TOPICAL voriconazole, TOPICAL natamycin, TOPICAL levofloxacin | Not available                     |                                   |
| [17]              |                        |                           |                          |                                      |                                   |                                   |
| Shivaprasaksh et al.| Vitreous tap        | *C. truncatum* (2)        | DNA sequencing            | Intradiscal amphotericin B, PPV; ± itraconazole, dexamethasone, TPK; ± oral and topical fluconazole | Not available                     | Clinical improvement; partial improvement (CF at 1.5 m) |
| [5]               |                        |                           |                          |                                      |                                   |                                   |
| Shiraishi et al.  | Corneal scrape          | *C. truncatum* (3), *Colletotrichum spp.* (1) | DNA sequencing | Topical voriconazole and natamycin; ± oral voriconazole; ± topical miconazole; ± topical levofloxacin | 2–3 weeks                         | Final Va = 20/16–20/300           |
| [12]              |                        | *C. gloeosporioides* (3)  | DNA sequencing            |                                      |                                   |                                   |

NLP, no light perception; TPK, therapeutic penetrating keratoplasty; PPV, pars plana vitrectomy

*a* Based on susceptibility data.

*b* Published minimal inhibitory concentration values.
important to look for microbiologic evidence of all possible etiologies of keratitis/endophthalmitis at the first visit, i.e. bacterial, viral and fungal. This will permit the rapid diagnosis and treatment of these infections, so as to protect sight and ensure the best outcome. A travel history may be helpful in considering unusual agents of infection. It is unclear whether species identification is necessary for the management of this infection, but it is important for the epidemiology of the disease. As the morphological identification of the agent is difficult, molecular techniques can help in its accurate diagnosis. With respect to management, a team approach involving ophthalmology, pathology, microbiology, and infectious diseases, is essential to ensure that the safest and most efficacious treatment is selected. There must be a high level of suspicion to achieve an early diagnosis of fungal keratitis. Once the diagnosis has been made, prompt treatment is necessary. Future studies are required to further understand the epidemiology and optimal management of the C. truncatum species complex.

Conflict of interest

CC has previously received honoraria from Allergan, Alcon Labs, and Bausch & Lomb. DW has previously received honoraria from Alcon/Novartis, Bausch & Lomb, Bayer, Labtician, Allergan. SR has previously received research grants from Astellas and Pfizer. YY, AA, NN, and VS have no financial disclosures. No funding was provided for this work.

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