A case of mycotic keratitis caused by *Colletotrichum,* a plant pathogen

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Abstract

Mycotic keratitis has emerged as a major ophthalmic problem. Fungal keratitis is usually caused by *Aspergillus, Fusarium, Curvularia* and *Candida* species. But one should be aware of other rare environmental pathogens causing keratitis. We report here two cases of corneal ulcer caused by plant pathogens *Colletotrichum truncatum* and *Colletotrichum gloeosporioides* following injury. Both the cases were treated successfully with voriconazole and natamycin.

Keywords*:* Corneal ulcer, *Colletotrichum truncatum*, *Colletotrichum gloeosporioides*, Plant Pathogen, Trauma.

Introduction

Advances in molecular biology techniques and newer diagnostic methods of infectious diseases have resulted in the control and eradication of many types of eye infections. Though, there is dramatic decrease in classical infections of the eye, new and emerging eye infections are on the rise. Environmental pathogens are a major concern in fungal keratitis following trauma. Case reports of *Colletotrichum spp*, a plant pathogen causing keratitis are on rise (Cannon et al., 2012; Cano et al., 2004; Damm et al., 2009). We report here a two cases of fungal keratitis caused by a plant pathogen *Colletotrichum truncatum and Colletotrichum gloeosporioides* which were treated successfully by voriconazole and natamycin.

Case Report

Case 1: A 54 year old man presented to ophthalmology outpatient department with complaints of pain, redness and gross diminution of vision in right eye since 7 days. His right eye was injured while removing the cobwebs at home few days back. There was no other significant history. He had consulted local ophthalmologist earlier and was treated with moxifloxacin eye drops and cycloplegics for 5 days. Slit lamp examination of the eye revealed dense central corneal infiltrate in the lower part of the cornea not extending to the limbus, measuring 3x5mm, intense ciliary congestion and a 1 mm dense hypopyon filling the anterior chamber. Dense slough was present at the base of the ulcer. Satellite lesions were absent. Vision was 6/60 unaided and 6/24 with pinhole. Other eye status was normal. Corneal scrapings and smears were sent for microbiological workup. 10% KOH mount showed septate branched hyphae and he was started on natamycin 5% drops and atropine 1% ointment QID. Patient was given moxifloxacin 400mg QID, Vitamin C 500mg daily and analgesics. Moxifloxacin was discontinued after negative bacterial culture report. As the patient was non-responsive to natamycin and fungal culture report received within 5 days showed fungal growth with unidentifiable features, treatment was changed to Voriconazole drops 1 drop hourly. Patient was followed up with regular weekly check ups for 4 months. During this time he showed a marked visual improvement but later he was lost for follow up.

Case 2: A 48 year old carpenter by profession presented with complaints of irritation, pain and blurred vision of the left eye since 10 days. He gave a history of wooden stick injury to the eye while working. On slit lamp examination, there was a small para-central corneal ulcer with 2 mm hypopyon of the left eye. The initial visual acuity of the affected eye was 6/60. Corneal scrapings were sent for microbiological workup. 10% KOH mount showed septate branched hyphae and he was started on voriconazole drops 1 drop hourly and atropine 1% ointment QID. A provisional diagnosis of fungal ulcer was made and voriconazole was started. The bacterial culture report was negative. Patient responded to treatment and fungal grew on culture after 5 days of incubation. Patient was followed up regularly for 8 months. On follow up there was a small para central opacity with a vision of 6/9 for which glasses were prescribed.

Both cases were treated as outpatients and no systemic antifungal were prescribed. They were treated with topical Voriconazole (vozol, aurolab) which is available as 30 mg /vial in a sterile lyophilised powder form. It is reconstituted with 3 ml of sterile water for injection to get 10 mg / ml(1% solution).The treatment was continued for 4 months in 1st case and 6 months for 2nd case.

Microbiological workup: Corneal scraping from the base of the ulcer was sent for gram stain, Potassium hydroxide (KOH) and fungal culture. Samples were inoculated on Sabouraud's dextrose agar (SDA), Sheep blood agar and incubated at 28^0C and 37^0C. KOH mount revealed septate, branched hyphae. Fungal growth on SDA had pinkish aerial mycelia that became grey-black with dark brown on the reverse (Fig. 1). Examination of the slide culture after 10 days of incubation showed septate, branched hyphae with

conidia and appressoria (Fig. 2). Conidia were hyaline, smooth-walled, aseptate and long. The central part was slightly curved with parallel walls, with round and truncated base and strongly curved apex. Appressoria were light brown, with the entire lobed edge having a roundish to ellipsoidal outline. The fungus was sent to Department of Mycology, Centre of Advance Research in Medical Mycology & WHO Collaborating Centre, Department of Medical Microbiology, Postgraduate Institute of Medical Education & Research (PGIMER) Chandigarh, India for further identification.

Fig. 1: Colony of *Colletotrichum truncatum*

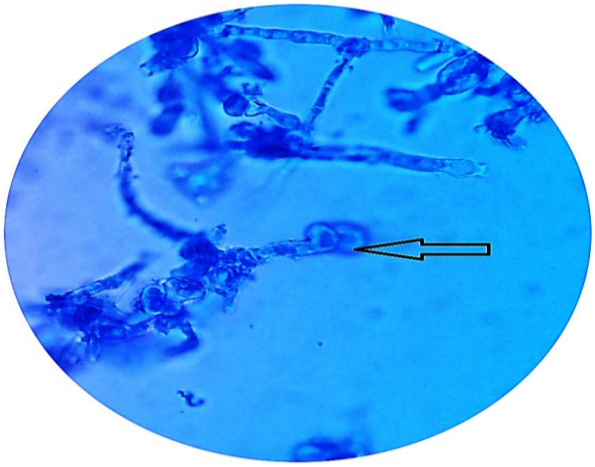

Fig. 2: Branched hyphae with conidia and appressoria of C*olletotrichum truncatum*

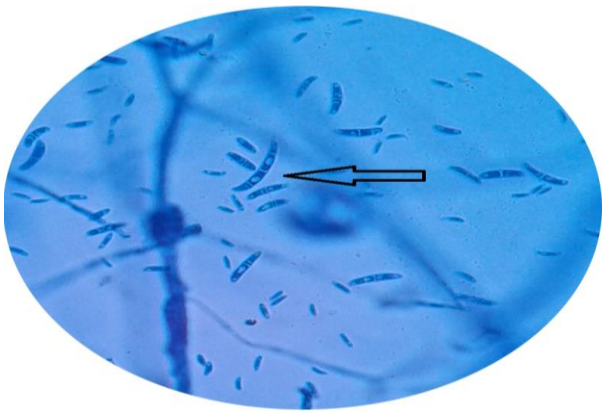

Fig. 3: Sepatae hyphae with macroconidia of *Colletotrichum gloeosporioides*

Molecular identification by sequencing of the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) identified it as *Colletotrichum truncatum* (Case 1). In Case 2, the corneal scrapings sent underwent same microbiological workup and showed septate hyphae with macroconidia (Fig. 4) microscopically. It was presumptively identified as *Colletotrichum* species based on conidial features and was confirmed as *Colletotrichum gloeosporioides* by molecular methods at PGIMER, Chandigarh.

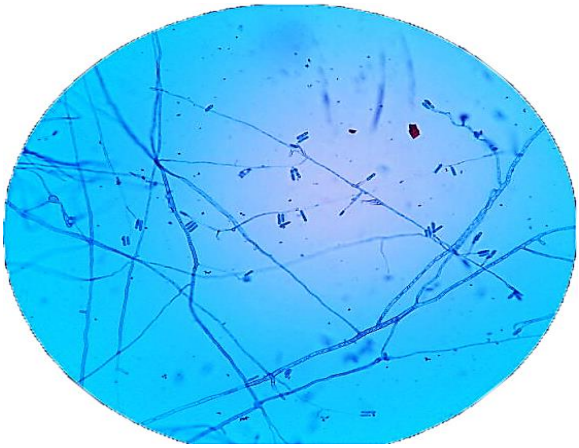

Fig. 4: Lactophenol cotton Blue mount of *Colletotrichum gloeosporioides* showing septate hyphae with macroconidia

Table 1: Review of previous reports of keratitis with *Colletotrichum species*

Mode of Infection	Author	Treatment	Outcome
Trauma with wooden stick	(Shivaprakash et al., 2011)	Therapeutic Keratoplasty, oral and topical fluconazole	Atrophic bulbitis with staphyloma
Subconjuctival injection of dexamethasone	(Shivaprakash et al., 2011)	Amphotericin B, Dexamethasone, itraconazole, pars plana vitrectomy	Clinical improvement
No trauma	(Shivaprakash et al., 2011)	Therapeutic keratoplasty	Clinical improvement
Insect fall	(Shivaprakash et al.,	Therapeutic keratoplasty	Corneal opacity

	2011)		
Trauma with wooden stick	(Shivaprakash et al., 2011)	Amphotericin B, Pars plana vitrectomy	Partial improvement in CF (1.5 m)
Cobweb fall while cleaning	Present case	Natamycin unresponsive changed to Voriconazole	Clinical improvement
Trauma with rotten wood	(Guarro et al., 1998)	-	-
Trauma with wooden chip	Present case	Voriconazole, 5% natamycin	Para-central leucoma
Keratitis following cataract	(Yamamoto et al., 2001)	Topical and systemic fluconazole for 2 months	Clinical improvement

Discussion

The genus *Colletotrichum* (*Glomerellaceae*, *Sordariomycetidae*, *Sordariomycetes*, *Ascomycota*) was described in 1831 by Corda, who provided drawings of *C. lineola* on a stem of an unidentified host belonging to the *Apiaceae* found in late autumn near Prague, Czech Republic (Gawade et al., 2009). The genus *Colletotrichum* consists of a number of plant pathogens of major importance, causing diseases of a wide variety of woody and herbaceous plants. It has tropical and subtropical distribution, although there are some high-profile species affecting temperate crops (Guarro et al., 1998) As plant pathogens, *Colletotrichum* species are chiefly known to cause anthracnose diseases, although other maladies are also reported such as red rot of sugar cane, coffee berry disease, crown rot of strawberry and banana, and brown blotch of cowpea (Lenne, 2009). In India, *Colletotrichum truncatum* is known to infect kharif crops such as paddy, sugarcane, groundnuts, maize, and a variety of pulses, which are grown during summer and rainy seasons (Natarajan et al., 2010) and *C. gloeosporioides* infects papaya, banana and avocado. In rare instances, *Colletotrichum* species have been implicated in human disease, causing keratitis and subcutaneous infections (Cannon et al., 2012; Cano et al., 2004; Shivaprakash et al., 2011).

Of 66 presently recognised species only few like *Colletotrichum dematium, Colletotrichum Coccodes, Colletotrichum Gloeosporioides, Colletotrichum Graminicola, Colletotrichum Crassipes, Colletotrichum capsicii* (currently named *Colletotrichum truncatum*) are known to cause human infections (Cannon et al., 2012). *Colletotrichum* produces falcate conidia which can be confused with *Fusarium*, a common fungus causing corneal ulcer. Hence it is important to identify the fungus not only for epidemiological purposes but also for treatment as antifungal susceptibilities differ for various species (Sutton, 1999). However, identification of *Colletotrichum* based on conidia, appressoria and acervuli grown on routine media in the diagnostic laboratory may be challenging for a microbiologist due to poorly defined and atypical characteristics displayed under artificial growth conditions. Conidia, appressoria and acervuli are better expressed when grown on potato dextrose agar or oatmeal agar (Cannon et al., 2012). In most of the time it needs to be sent to reference laboratory for identification due to the non-familiarity of the fungus among medical microbiologists.

Owing to the complexity involved in the morphological identification of *Colletotrichum* spp. in the diagnostic laboratory, the molecular approach seems to be more appropriate. Phylogenetic analysis based on the nucleic acid sequence of the internal transcribed spacer region of the rDNA is the most suitable method for the identification of *Colletotrichum* isolates to the species level (Cannon et al., 2012; Yamamoto et al., 2001). This is more significant for a epidemiological purposes, apart from the fact that most of the strains of *Colletotrichum trunactum* from India are misidentified as *Colletotrichum dematium* based on morphological identification due to their close resemblance (Cannon et al., 2012) also noticed elsewhere (Sutton, 1999) In our study identification of C*olletotrichum truncatum* was made by molecular sequencing.

In the absence of molecular method correct identification based on morphology is very difficult for an untrained eye and hence an incorrect identification as *Fusarium* species. Natamycin has been the traditional drug for the treatment of *Fusarium* mycotic keratitis cases, but inactive in *Colletotrichum truncatum* and may lead to worsened prognosis and even corneal blindness.(Yamamoto et al., 2001) In our centre, natamycin 5% is the drug used by ophthalmologists for filamentous mycotic keratitis but since patient was non-responsive and based on fungal growth and morphology, treatment was shifted to Voriconazole. Though antifungal susceptibility testing is recommended for *Colletotrichum infections,* it could not be carried out due to lack of facilities in our laboratory.

Like other ophthalmic infections, *Colletotrichum* infections are best managed by combination of medical and surgical treatment such as total penetrating keratoplasty keratitis or pars plana vitrectomy for endophthalmitis. (Cannon et al., 2012) In vitro antifungal susceptibility data suggests that empiric therapy with amphotericin B in deep, invasive disease is warranted. A combination of topical antifungals

along with oral triazole agents appear effective in superficial /subcutaneous settings (Yegneswaran et al., 2010) Table 1. Shows comparison of different *Colletotrichum truncatum* and *Colletotrichum. Gloeosporioides* cases in terms of mode of infection, treatment and outcome.

Fusarium like fungus not responding to natamycin in an event of vegetative trauma should raise the suspicion of *Colletotrichum* infections and treatment should immediately be shifted to azoles such as voriconazole eye drops. Accurate identification of the fungus with early initiation of therapy can help in total recovery.

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