Original Article

Molecular identification of *Podospora bulbillosa* keratitis with progressive stromal thinning: a case report

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ABSTRACT

Fungal keratitis (FK) is a sight-threatening corneal infection that is particularly prevalent in developing countries. This report describes a case of *Podospora bulbillosa* keratitis, a rare organism confirmed by polymerase chain reaction (PCR) following negative culture results. A 63-year-old male presented with a well-demarcated yellow-brown stromal infiltration. Microbiological evaluation revealed pigmented septate hyphae; however, culture remained negative, and molecular identification was performed using ITS1-5.8S-ITS2 rDNA sequences, which identified the isolate as *Podospora bulbillosa* (formerly *Cladorrhinum bulbillosum*). The patient received intensive topical voriconazole 1% and chlorhexidine 0.2% combined with oral voriconazole. Progressive thinning required cyanoacrylate glue. The infection resolved over three months, leaving a central scar. While infections by this organism have been rarely reported, additional cases are crucial to consolidate knowledge of clinical presentation and potential aggressiveness. This case emphasizes the value of molecular diagnostic tools in detecting unusual pathogens and guiding therapy.

KEYWORDS
Cladorrhinum bulbillosum, Fungal keratitis, PCR, Voriconazole.

INTRODUCTION

Fungal keratitis (FK) is a significant cause of corneal ulceration, particularly in tropical and subtropical regions (Sharma et al. 2022). Diagnosis and management remain challenging because clinical manifestations are variable, microbiological confirmation may be delayed, and therapeutic response is often prolonged (Atighehchian et al. 2025a, Tawde et al. 2023).

The most frequently isolated organisms include *Fusarium*, *Aspergillus*, and *Candida* species (Ghenciu et al. 2024). In contrast, infections caused by uncommon fungi are poorly characterized, and their clinical behavior and optimal management are not well defined (Atighehchian et al. 2025b). Polymerase chain reaction (PCR) has emerged as a sensitive tool for detecting fungal DNA. It is especially valuable when cultures are negative or rare pathogens are suspected (Khalili et al. 2020, Atighehchian et al. 2025c)

Additional reports of uncommon fungi are vital to improve epidemiologic understanding, clinical behavior and alert ophthalmologists to the early diagnosis of these uncommon pathogens (Khalili et al. 2020). Here, we describe a case of

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keratitis caused by *Podospora bulbillosa* (formerly *Cladorrhinum bulbillosum*), an infrequently reported saprophytic fungus, and discuss its presentation, clinical course, and response to therapy.

MATERIALS AND METHODS

A 63-year-old male farmer was referred to our hospital with a six-day history of decreased vision, ocular pain, redness, and photophobia in his left eye. Fourteen days before his presentation, he had accidental exposure to an agricultural chemical spray. His systemic medical history was unremarkable. In this case, best-corrected visual acuity (BCVA) was measured, the anterior segment was examined by slit-lamp biomicroscopy, and posterior segment involvement was assessed using B-scan ultrasonography. Corneal scrapings were aseptically obtained from the base and margins of the ulcer for microbiological investigation. Direct microscopic examination was performed using a 10% potassium hydroxide (KOH) wet mount to detect fungal elements. The specimens were subsequently inoculated onto Sabouraud dextrose agar (SDA) and incubated under standard laboratory conditions for fungal culture (Ghenciu et al. 2024).

Because direct microscopy demonstrated fungal hyphae while repeated culture remained negative, a corneal biopsy specimen was obtained for molecular identification (Tawde et al. 2023). Genomic DNA was extracted from the biopsy specimen using a commercial fungal DNA extraction kit (Gene All, Germany), according to the manufacturer's instructions. The internal transcribed spacer (ITS) region of the ribosomal DNA, including ITS1, the 5.8S rDNA gene, and ITS2, was amplified by polymerase chain reaction (PCR) using universal fungal primers. The PCR product was purified and subjected to Sanger sequencing. The PCR was performed in a 50 µl reaction mixture containing diluted genomic DNA in optimum concentration, 0.5 µM of each primer, 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 0.08% Nonidet P40, 1.5 mM magnesium chloride (MgCl2), 200µM each of nucleotide triphosphate and 2 units Taq DNA polymerase. The reaction was performed for 35 cycles: denaturation at 93°C for 1 min, annealing at 52°C for 50 sec, extension at 72°C for 1 min and 20 sec, with an initial denaturation at 94°C for 1 min before cycling and final extension at 72°C for 10 min after cycling. The obtained sequence was compared with sequences available in the National Center for Biotechnology Information (NCBI) GenBank database using the Basic Local Alignment Search Tool (BLAST) to determine the fungal species (Atighehchian et al. 2025b, Ghenciu et al. 2024).

The patient was managed according to the institutional protocol for fungal keratitis, and treatment was modified based on microbiological and molecular findings as well as the clinical response during follow-up (Atighehchian et al 2025).

RESULTS

A 63-year-old male farmer presented with a six-day history of decreased vision, ocular pain, redness, and photophobia in his left eye. Fourteen days before presentation, he had accidental exposure to an agricultural chemical spray. His medical history was unremarkable, and he had no history of systemic immunosuppression. The BCVA in the affected eye was 1.0 LogMAR. Slit-lamp examination revealed mild conjunctival injection and a well-demarcated 5 × 6 mm mid-stromal corneal infiltrate with yellow-brown pigmentation (Fig. 1).

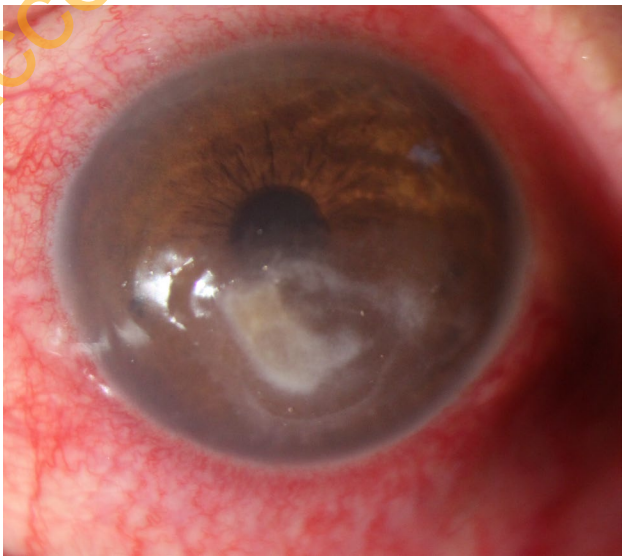

Fig. 1. The slit-lamp photograph shows a 5 × 6 mm infiltration with a central pigmented area and well-defined margins.

No hypopyon or stromal melting was observed at presentation. B-scan ultrasonography demonstrated no posterior segment abnormalities. The patient was admitted and initially treated empirically with hourly fortified amikacin 2.5% (25 mg/mL) and cefazolin 5% (50 mg/mL), as fungal keratitis was not initially suspected. Direct microscopic examination of corneal scrapings using a 10% potassium hydroxide (KOH) preparation revealed pigmented septate hyphae (Fig. 2), consistent with filamentous fungal keratitis. The rDNA-ITS sequence analysis showed 99.7% identity (two substitutions and two gaps) with the ITS sequence of type material of *Podospora bulbillosa* deposited in GenBank under accession number NR077199 (Isolate: CBS 304.90). Accordingly, the fungus was identified as *Podospora bulbillosa* (formerly *Cladorrhinum bulbillosum*). The sequence was deposited in GenBank under accession number PZ704156.

Fig. 2. Direct examination performed using potassium hydroxide (KOH) preparation revealed the presence of pigmented septate hyphae.

Accordingly, antibacterial therapy was discontinued, and treatment was changed to hourly topical voriconazole 1%, topical chlorhexidine 0.2% every two hours, oral voriconazole 200 mg twice daily, and topical levofloxacin 0.5% four times daily. Natamycin was unavailable, and liver enzymes were monitored twice weekly. By day 5, early peripheral scarring was observed, but progressive central thinning developed (Fig. 3).

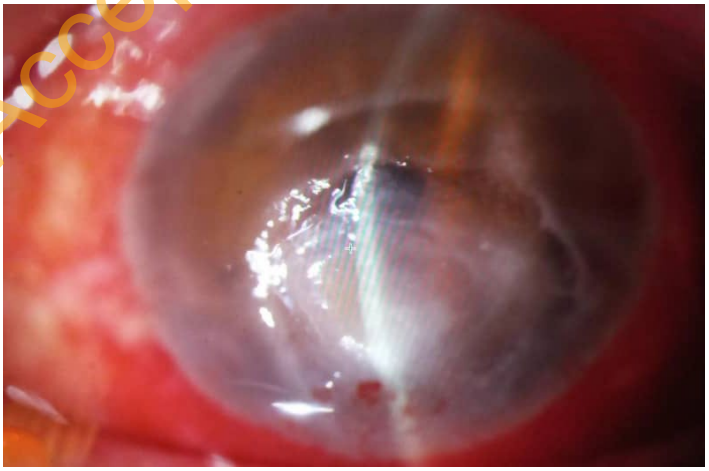

Fig. 3. On the 7th day after starting voriconazole 1% and cholohexdin 0.2%, the slit-lamp photograph shows corneal stromal thinning had progressed.

Culture on Sabouraud dextrose agar remained negative after seven days. Therefore, on day 7, intrastromal voriconazole (50 µg/0.1 mL) was administered. Cyanoacrylate glue was applied to prevent perforation, and antifungal therapy was continued (Fig. 4).

Molecular analysis of the corneal biopsy specimen identified the causative organism as *Podospora bulbillosa***.** The patient was discharged and continued topical voriconazole and chlorhexidine every four hours. Gradual resolution of

the infiltrate occurred. Three months later, the glue was removed, leaving a dense central scar with superficial vascularization. Final visual acuity improved to 0.4 LogMAR. (Fig. 5).

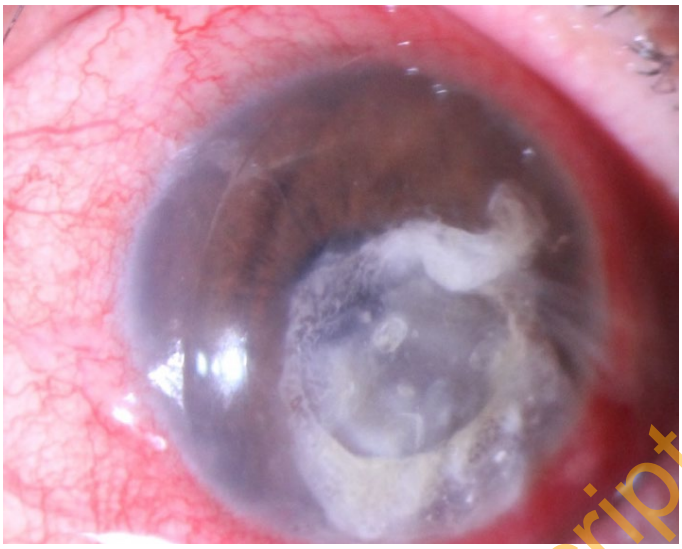

Fig. 4. Patient underwent cyanoacrylate glue application, and antifungal medication was continued.

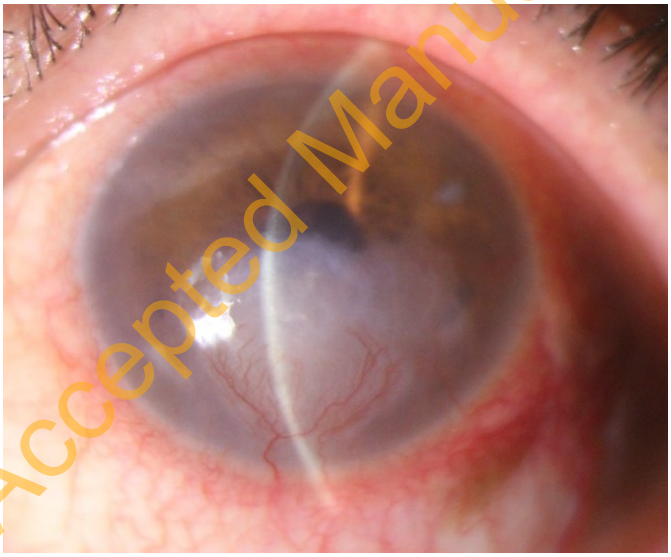

Fig. 5. Slit-lamp photograph obtained at the three-month follow-up after cyanoacrylate glue removal, demonstrating resolution of the corneal infiltrate.

DISCUSSION

Podospora species are environmental saprobes commonly found in soil and plant debris (Madrid et al .2011). This filamentous fungus belongs to the dematiaceous group and can produce melanin, which may affect its clinical appearance and result in pigmentation (Zapater and Scattini 1979, Gajjar et al. 2011). Corneal ulcers caused by this genus have been reported; however, the total number of reported cases remains small (Gajjar et al. 2011, Natarajan and Mehta 2020). Consequently, the spectrum of clinical manifestations, virulence potential, and optimal therapeutic strategies has not yet been fully defined (Gajjar et al. 2011).

Taxonomic classification of this organism has recently changed (Wang et al .2019). Based on multilocus phylogenetic analyses of the family *Podosporaceae*, *Cladorrhinum bulbillosum* was transferred to the genus *Podospora* and is currently recognized as *Podospora bulbillosa*. Consequently, previously published ophthalmic reports describing *Cladorrhinum bulbillosum* keratitis refer to the same fungal species under its former nomenclature. We therefore use the currently accepted taxonomic name while acknowledging the historical designation to facilitate comparison with earlier literature (Wang et al. 2019).

Pigmentation of the corneal infiltrate is not pathognomonic, and it may serve as an important clinical clue for certain fungal species. For example, *Curvularia* keratitis is characteristically associated with brown to black discoloration of the infiltration (Narayanan et al. 2023). Previous studies have also documented stromal pigmentation in dematiaceous fungal infections, such as, *Podospora bulbillosa* (formerly *Cladorrhinum bulbillosum*), which can vary in intensity and distribution (Ghosh et al. 2020, Mitra et al. 2024). Gajjar et al. (2011) reported a severe case of pigmented *C. bulbillosum* keratitis that progressed to corneal perforation. In contrast, Narayanan et al. (2023) have described different ring-shaped presentations. These findings highlight the heterogeneous clinical spectrum of this rare organism (Natarajan and Mehta 2020). Our observation of a brown, well-circumscribed infiltrate aligns with the broader pattern of dematiaceous keratitis.

Gajjar et al. (2011) documented a severe case of pigmented *C. bulbillosum* keratitis, which led to corneal perforation, and attributed the poor outcome partly to the patient's systemic immunosuppression. In contrast, our patient, who was not immunocompromised, experienced rapid progression of the infection to stromal thinning within the first two weeks. This suggests that, although the patient had a competent immune system, the organism may demonstrate aggressive local behavior independent of the host's immunity.

Microbiological evaluation remains the standard approach for the diagnosis of infectious keratitis; however, its sensitivity may be limited, particularly in partially treated eyes or when fastidious organisms are involved (Sharma et al. 2022, Atighehchian et al. 2025a). In addition, culture techniques often require five to seven days for fungal organisms to yield results (Atighehchian et al. 2025b). In the present case, cultures remained negative after 7 days, whereas PCR enabled rapid and definitive identification of *P. bulbillosa*. These molecular techniques can detect uncommon pathogens and prevent the under-recognition of rare fungi (Tawde et al. 2023). Our findings also support integrating PCR into the diagnostic algorithm for suspected fungal keratitis, especially when routine microbiological methods are inconclusive.

The therapeutic experience with *Podospora bulbillosa* showed positive outcomes with treatments such as natamycin, amphotericin B, and azole derivatives; however, some cases have been aggressive and have required keratoplasty (Ghosh et al. 2020, Buchta et al. 2019). In our patient, we achieved infection control using intensive topical and systemic voriconazole combined with chlorhexidine. This treatment regimen has rarely been documented for this species and may serve as a practical alternative, especially in areas where natamycin is not available. Additionally, we found that using cyanoacrylate glue for tectonic support was necessary to maintain the eye's integrity.

CONCLUSION

We present the first molecularly confirmed case of keratitis caused by *Podospora bulbillosa* in Iran. Although this organism is rare, it can cause significant stromal destruction even in immunocompetent patients. The early use of molecular diagnostics, coupled with intensive antifungal treatment and timely surgical intervention, can preserve the eye. Each additional molecularly documented report enhances understanding of the organism's epidemiology, refines its clinical presentation, and increases clinical suspicion.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTION

Sadegh Khodavaisy, Alireza Izadi, Zohreh Abedinifar: Culture of specimen, microscopic examination, original draft preparation; Mehrnaz Atighehchian, Koosha Koorehpaz, Mehrnaz Zarei-Ghanavati: Identification, rDNA analysis, review and editing.

DATA AVAILABILITY

All data are available in online repositories. Requests for additional data and materials should be addressed to Mehrnaz Atighehchian.

FUNDING

Not applicable.

DECLARATION

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

Not applicable.

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قارچ شناسی ایران جلد ۱۳، شماره ۱، ۱۴۰۵

مقاله کامل

شناسایی مولکولی عفونت قرنیه ناشی از *Podospora bulbillosa* همراه با نازک شدگی پیشرونده استروما: گزارش یک مورد

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چکیده

کراتیت قارچی یک عفونت تهدیدکننده قرنیه است که به‌ویژه در کشورهای در حال توسعه شیوع بالایی دارد. در این گزارش، یک مورد کراتیت ناشی از *Podospora bulbillosa* به‌عنوان یک عامل بیماری‌زای نادر که پس از منفی بودن نتایج کشت، با استفاده از واکنش زنجیره‌ای پلیمراز (PCR) تأیید شد، توصیف می‌شود. بیمار، مردی ۶۳ ساله، با یک عفونت قرنیه زرد-قهوه‌ای با حدود مشخص مراجعه کرد. بررسی‌های میکروبیولوژیک وجود هیف‌های بند بند رنگی را نشان داد، اما کشت قارچی منفی بود. ازاین‌رو، شناسایی مولکولی با تعیین توالی ناحیه ITS1-5.8S-ITS2 rDNA انجام شد که عامل بیماری را به‌عنوان *Podospora bulbillosa* (که پیش‌تر با نام *Cladorrhinum bulbillosum* شناخته می‌شد) شناسایی کرد. بیمار تحت درمان با وریکونازول موضعی ۱٪ و کلرهگزیدین موضعی ۰/۲٪ به‌صورت فشرده، همراه با وریکونازول خوراکی قرار گرفت. به دلیل نازک‌شدگی پیشرونده قرنیه، از چسب سیانو آکریلات استفاده شد. عفونت طی سه ماه برطرف شد، اما یک اسکار مرکزی در قرنیه باقی ماند. اگرچه عفونت‌های ناشی از این قارچ به‌ندرت گزارش شده‌اند، گزارش موارد بیشتر برای تکمیل دانش موجود درباره تظاهرات بالینی و میزان بالقوه تهاجمی بودن آن ضروری است. این مورد، اهمیت استفاده از روش‌های تشخیص مولکولی را در شناسایی عوامل بیماری‌زای غیرمعمول و انتخاب روشهای درمان مناسب را برجسته می سازد.

واژگان کلیدی: پی سی آر، کراتیت قارچی، وریکونازول، *Cladorrhinum bulbillosum*.

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