CASE REPORT

Rare Fungal Keratitis Caused by *Coprinellus Radians*

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Abstract A case of fungal keratitis due to *Coprinellus radians* is reported. To our knowledge, fungal keratitis caused by this species was rare. Fungal hyphae were detected in corneal scrapings, and isolates were identified by morphology and by sequencing the internal transcribed spacer region of ribosomal DNA. The patient was treated with systemic and local antifungal therapy for 5 days, and lamellar keratoplasty was performed after no obvious improvement in symptoms. The in vitro antifungal susceptibilities of the case strain were tested for six antifungal agents. The results showed that 5-fluorouracil was resistant, fluconazole was moderately sensitive, and the other drugs assayed (amphotericin B, posaconazole, itraconazole and voriconazole) were highly effective against this fungus.

Keywords Fungal keratitis · *Coprinellus radians* · In vitro antifungal susceptibility test

Introduction

At present, more than 100 species that belong to 50 genera of fungi pathogens have been reported which cause human corneal infection [1]. The pathogens of fungal keratitis vary in different climatic regions. Filamentous fungi are the main pathogens throughout the world, especially in tropical and subtropical areas, and the most common pathogens are *Fusarium* and *Aspergillus* [1–5]. In China, the most common pathogenic agents are *Fusarium*, *Aspergillus* and *Alternaria* [6]. As a basidio-mycotic fungus, *C. radians* which is commonly known as a contaminant has not been reported as the cause of this infection. Therefore, we describe a case of keratitis due to *C. radians*.

Case Report

A 48-year-old female farm worker, her left eye was scratched by corn leaves 40 days ago, complained of foreign body sensation, with vision loss, lacrimation, photophobia and no other obvious discomfort. She was diagnosed with a corneal ulcer at local hospital and was given topical antibiotic eyedrops (specific medication is unknown), significant improvement in symptoms of self-discomfort. Two weeks later, her condition deteriorated, the symptoms such as eye abrasion and vision loss were significantly aggravated, accompanied by difficulty in opening eyes, so she

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went to the local hospital again and received systemic and topical anti-infection treatment, but because of no effect, she was referred to our hospital.

On first examination, the patient's vital signs were normal and her existing spectacle correction visual acuity was FC/20 cm in the left eye, which showed diffuse conjunctival hyperemia. There was a 5 mm × mm gray–white infiltration with irregular borders and edema in the center cornea, invasion of shallow and middle stroma (Fig. 1a). No abnormality was observed in the right eye.

On image logical examinations, anterior segment coherence optical tomography (AS-OCT) (Optovue, USA) showed that the central cornea infiltration depth was about 100 μm and in vivo confocal microscopy (IVCM) (Heidelberg HRT3, RCM) showed that the accumulation of medium amount of necrotic material

and the invasion depth of hyphae structure reached 200 μm , with a large number of chlamydospore-like structures clustered (Fig. 2). The endothelial cell count was about 2269 / mm^2 .

Corneal scraping was performed at the bottom and edge of corneal ulcer, and placed on a microscope slide which drip 10% potassium hydroxide; it showed abundant thick hyaline septate and branched hyphae, with abundant chlamydospore in the middle or terminal of the hyphae (Fig. 3a), while other scrapings were directly inoculated into 5% sheep blood agar and Sabouraud glucose agar (SDA), and incubated at 28 °C and 35 °C, respectively. After 2–3 days, small white colonies, apparently of a single fungus species, appeared on all the culture media.

After hyphae were detected, the patient was given voriconazole 10 mg/ml eyedrops four times per hour,

Fig. 1 **a** Slit-lamp examination of the left eye showing a 5 × 5 mm² gray–white lesion infiltrate at the center cornea, invasion of shallow and middle stroma, **b** lamellar keratoplasty after ineffective antifungal therapy

Fig. 2 Confocal microscopy, **a** a large number of chlamydospores are scattered in chains, **b** a large number of hyphae are densely distributed

Fig. 3 **a** 10% potassium hydroxide smear showed a large number of complete hyphae, which were thick, septate, middle or terminal chlamydospore, solitary, chain-shaped or clustered-

shaped (magnification of $\times 200$), **b** gram staining showed that the hyphae were wide and irregular in width (magnification of $\times 1000$)

Fig. 4 **a** Colony morphology of SDA medium cultured at 28 °C for 8 days showed that the colony was fluffy cotton flocculent and dark yellow in the middle, light yellow around, and **b** the reverse was yellow and yellow-brown. (Color figure online)

amphotericin B 0.25% eyedrops once an hour, levofloxacin 0.5% eyedrops four times a day, pranoprofen 0.1% eyedrops four times a day and ofloxacin 0.3% eye ointment once a night. Five days later, the inflammation did not subside. In order to avoid missing the operation opportunity, partial lamellar keratoplasty (glycerin preserved corneal tissue) was performed under peribulbar anesthesia after the patient signed the informed consent. After surgery, there was therapy with voriconazole 10 mg/ml eyedrops once an hour, amphotericin B 0.25% eyedrops every two hours, ofloxacin 0.3% eye ointment four times a day and recombinant bovine basic fibroblast growth factor eye gel four times a day. Five days later, the voriconazole and amphotericin B 0.25% eyedrops were decreased to four times daily; at the

same time, levofloxacin and pranoprofen eye drops were given four times a day. Eight days after the surgery, the corneal ulcer healed and the patient was discharged from hospital (Fig. 1b), and her visual acuity with spectacle correction improved to 20/200. The topical antifungals were tapered and discontinued, and outpatient follow-up was conducted.

Mycology

We identified fungi by morphology and molecular methods. The clinical isolate was subcultured on SDA and incubated in 28 °C. After 8 days, the colonies on SDA were floccose, with dark yellow fluffy aerial mycelia in the middle, light yellow around and the

Fig. 5 SDA medium was cultured at 28 for 8 days and stained with lactophenol cotton blue staining (**a** magnification of $\times 400$, **b** magnification of $\times 1000$)

Table 1 A checklist for submission of case report to Mycopathologia

	Yes	No
Original observation	☒	☐
Ethical statement included	☒	☐
Patient demographic anonymized	☒	☐
Title includes the term for medical and veterinary condition being described and the correct name of the fungal pathogen (italicized)	☒	☐
Title and abstract do not include superlatives such as 'new', 'first', 'unique', etc.	☒	☐
Patient treatment and follow-up included	☒	☐
Confirmatory, probable or possible diagnostic criteria defined Mycology	☒	☐
Any author a specialist in fungal diagnostics?	☒	☐
Culture positive with characteristic microscopy	☒	☐
Culture confirmed with molecular tests	☒	☐
Fungus name conform to current nomenclature	☒	☐
Nucleotide sequence deposited in a known database	☒	☐
Antifungal susceptibility test results included	☒	☐
Antifungal test result interpretation provided with reference to the guidance documents (CLSI, EUCAST)	☒	☐
Histopathology		
Any author a specialist in histopathology?	☒	☐
High-resolution images included	☒	☐
Specialized fungal stains used	☒	☐
Differential histopathology diagnosis discussed	☒	☐

Table 2 Mycopathologia IMAGES checklist

Original, unpublished observation being reported with ethical statement	☒	☐
Report of potential interest to a mixed audience of physicians and laboratory scientists	☒	☐
Brief title (three-four keywords)	☒	☐
One paragraph description not to exceed 500 words	☒	☐
No abstract, literature citation, bibliography or hyperlinks	☐	☒
Maximum of four images with short legends (colored required for free online publication)	☐	☒
Author and primary affiliation at the end of the paragraph (name, degree, primary institution name, city, state, country)	☒	☐

reverse was yellow and yellow–brown (Fig. 4). Microscopically, the hyphae were transparent, septated and branched. Conidiophore on both sides and tip of hyphae were short and thick. The conidia were single-celled, the ends were blunt, sausage-like, S-shaped and C-shaped and clustered in slimy head (Fig. 5).

According to its morphological and microbial characteristics, it was preliminarily identified as *Coprinellus* spp.; however, the genus could not be ascribed to which species. To confirm the identification, the internal transcribed spacer (ITS) region was sequenced and DNA sequences were determined at Kingdom clinical trial center (Guangzhou, China). The resulting sequences were deposited in the GenBank database. Species identification was performed by searching databases using the BLAST sequence analysis tool (<https://www.ncbi.nlm.nih.gov/BLAST/>). The ITS sequence of the isolate was 99.69% similar to that of strain *C. Radians*.

Antifungal Susceptibility Testing

In vitro antifungal susceptibility testing was performed by Sensititre Yeast One™ (AccuMed International, Chicago, IL, USA) [7–10]. The isolate was subcultured on PDA plates and incubated at 28 °C for 7 days. The plates were covered with 0.85% saline, followed by gentle pipetting of the colonies with the tip of a sterile pipette, and adjustment of the concentration of suspension to 0.5 Maxwell turbidity. The incubator temperature was 28 °C, and reading results

after 48 h. The minimal inhibitory concentrations (MICs) for fluconazole, itraconazole, voriconazole, posaconazole, 5-fluorocytosine and amphotericin B were 16, 0.25, 0.015, 0.25, > 64 and 0.25 µg/ml, respectively (Table 1).

Discussion

Coprinellus radians belongs to large mushrooms in nature, subordinate to Basidiomycota, Agaricomycetes, Agaricomycetidae, Agaricales, Psathyrellaceae and Coprinellus.

Although the relevant information of *C. radians* was available in the previous literature, most of them were isolated from sputum specimens, diabetic feet, abdominal drainage fluid and skin ulcer wounds, which could not exclude the possibility of contamination, and there were few clinical reports of eye infection caused by this genus. The patient's left eye was scratched by corn leaf and had a long course of disease. Slit-lamp examination showed a gray–white ulcer lesion of about 5 mm × 5 mm in the cornea central pupil area. Combining with corneal scrape histology, IVCN and culture results, it conformed to the predisposing causes, clinical manifestations and etiological diagnosis of ocular fungal infection.

Coprinellus radians has low nutritional requirements and can grow on a variety of media, the most suitable medium is SDA and PDA, and the culture time is about 10 days. The colony characteristics were obvious and grew rapidly on SDA medium. Its color is bright and like cotton flocculent, the middle is fluffy,

dark yellow, and the surrounding is light yellow as well as yellow or yellow–brown on the back. The color gradually disappeared after many generations. The spores are single-celled, the ends are blunt, sausage-like, S-shaped and C-shaped and clustered in slimy head. Although the colonies and spores of the genus are distinctive and easy to identify, they should be distinguished from those of some *Fusarium* species in order to avoid confusion (Table 2).

In vitro drug sensitivity test showed that the MIC values of amphotericin B, voriconazole, posaconazole and itraconazole were lower, while the MIC values of fluconazole and 5-fluorocytosine were higher. Due to the lack of a unified drug sensitivity break point, it is not yet possible to determine whether drug resistance exists. However, according to the MIC values, the rare genus was highly sensitive to amphotericin B, itraconazole, posaconazole and voriconazole, and resistant to fluconazole. Whether there is intrinsic drug resistance has not been reported. The patient was first treated in a primary hospitals and its specific medication was not clear; when she came to our hospital, the lesion had invaded the stroma, and sensitive antifungal agents and timely lamellar keratoplasty are effective. Therefore, fungal keratitis needs early diagnosis to identify different fungi genera and symptomatic treatment.

In summary, to our knowledge, this case of mycotic keratitis represents the first known reported infection caused by *C. Radians*, as well as being the first known reported in humans caused by this rare species.

We provided the checklist according to the request of the magazine [11].

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest and no ethical issue.

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