

Characterization of *Humicola pulvericola* First Isolated from a Patient with Keratitis Clinical Case

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Abstract: Infection caused by the *Humicola* sp is extremely rare. We report the first case of fungal keratitis caused by *Humicola pulvericola* (*H. pulvericola*) in a 63-year-old man with a history of exposed to hot oil two weeks ago who developed keratitis. Direct examination of confocal microscopy and corneal scrapings showed fungal hyphae and isolates were identified by morphology and by sequencing the internal transcribed spacer region of ribosomal DNA. The in vitro antifungal susceptibilities of the case strain were tested for five antifungal agents. The results showed that the infectious agent was resistant towards fluconazole, caspofungin and amphotericin B; itraconazole and voriconazole were highly effective against *H. pulvericola*. He was diagnosed with *H. pulvericola* keratitis and treated with oral itraconazole and natamycin eyedrops. After one month of treatment, the lesion gradually improved, with the best-corrected visual acuity improving to 0.8.

Keywords: *Humicola pulvericola*, keratitis, itraconazole, fungal infection, antifungal drugs

Introduction

Fungal aetiology is widely recognized as the primary culprits responsible for ocular morbidity, and *Fusarium* and *Aspergillus* are the most commonly encountered pathogens, especially in tropical and subtropical areas.^{1–3} *Humicola* is a genus of hyphomycetes related to the family *Chaetomiaceae*, which frequently found in various environments such as soil, indoor spaces, and compost habitats.⁴ Within this genus, more than 50 different species have been identified and described.⁴ However, it is worth noting that human infections caused by *Humicola* species are extremely rare and have seldom been reported in scientific literature.⁵ Here, we report first case of fungal keratitis caused by *H. pulvericola* in a 63-year-old man with a history of exposed to hot oil two weeks ago who developed keratitis.

Case Presentation

A 63-year-old man presented with pain, redness, and foreign body sensation in his right eye after being exposed to hot oil two weeks ago. His best-corrected visual acuity in the right eye was 0.2. Obvious conjunctival congestion, with white opacity on the temporal side of the cornea and visible brown foreign body in his right eye (Figure 1A). Confocal microscopy revealed abundant hyphae structure at the lesion of the right cornea (Figure 1B). No abnormality was observed in the left eye. Calcofluor white staining of corneal scrapings showed abundant septate hyaline (Figure 1C). Corneal 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 the two culture media.

The clinical isolate was subcultured on potato dextrose agar (PDA) and SDA, and incubated at 25°C and 35°C in the dark. After 7 days incubated at 25°C, the colonies on PDA and SDA were fluffy, with pale yellow aerial mycelia. At 35°C incubated 7d, the colony on PDA was yellow-brown (obverse), and a dark black color (reverse); colony on SDA was grey (obverse), and a dark black color (reverse) (Figure 2A). Micromorphology examination showed hyphae hyaline

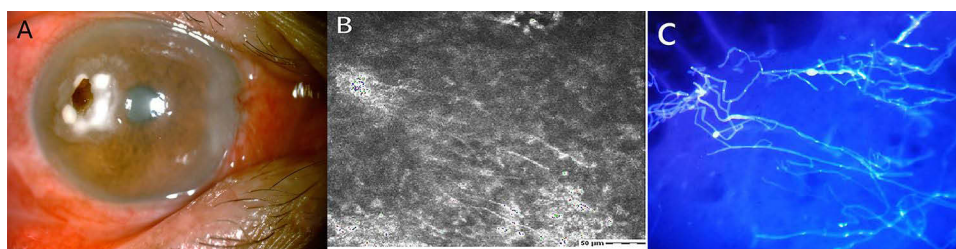


Figure 1 (A) Obvious conjunctival congestion, with white opacity on the temporal side of the cornea and visible brown foreign body in his right eye; (B) Confocal microscopy revealed abundant hyphae structure at the lesion of the right cornea; (C) Calcofluor white staining of corneal scrapings showed abundant septate hyphae.

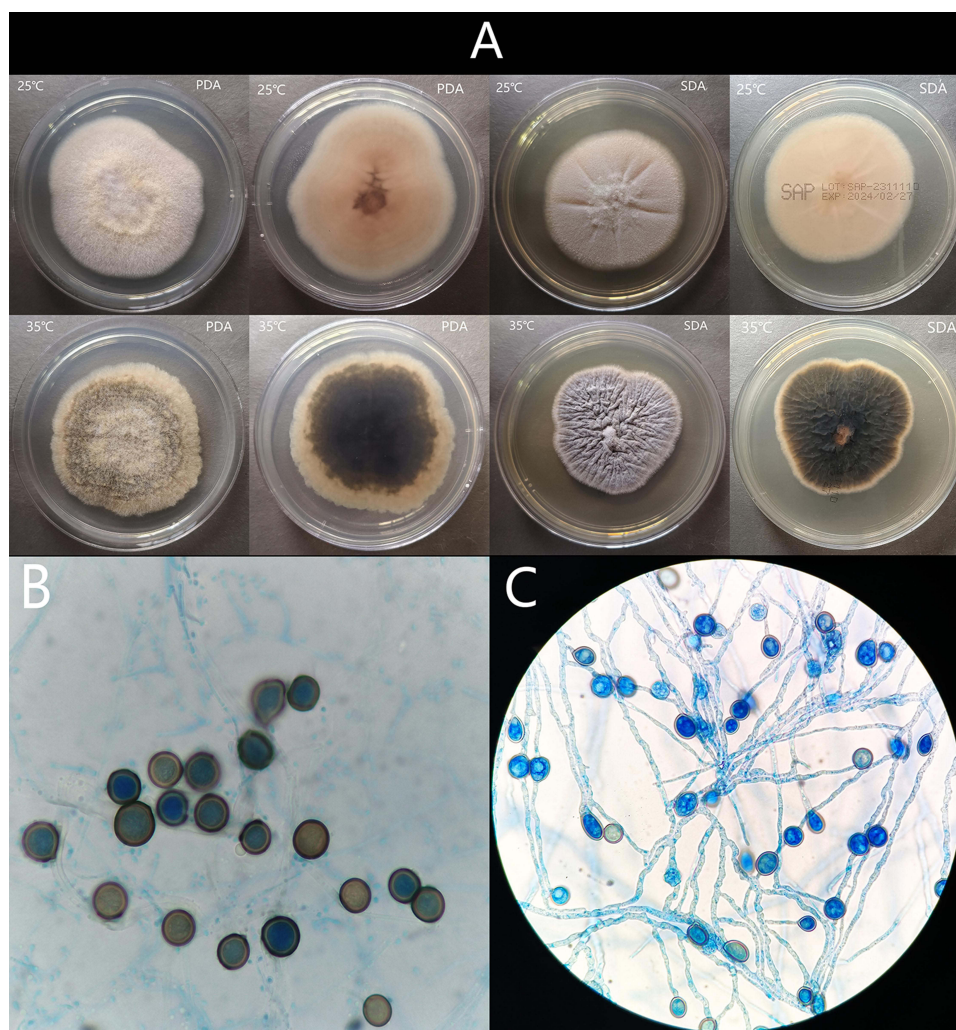


Figure 2 (A) The colonies on PDA and SDA after 7 days incubated at 25°C and 35°C; (B and C) Micromorphology examination showed hyphae hyaline and chlamydospores. Chlamydospores produced laterally or intercalary on hyphae, single-cell, thick-walled, brown, globose to subglobose.

and chlamydospores. Chlamydospores produced laterally or intercalary on hyphae, single-cell, thick-walled, brown, globose to subglobose (Figure 2B and C). It progressively became darken after 14 days and aerial hyphae gradually enrich (Figure 3A). Micromorphology examination showed two types of conidia: chlamydospores and acremonium-like conidiophores (Figure 3B). Acremonium-like conidiophores, which were small ($2.5\text{--}3\text{ }\mu\text{m} \times 1.2\text{--}1.8\text{ }\mu\text{m}$), hyaline, aseptate, smooth, obovoid, with slightly truncate ends, arranged in long divergent chains on the apex of the flask shaped

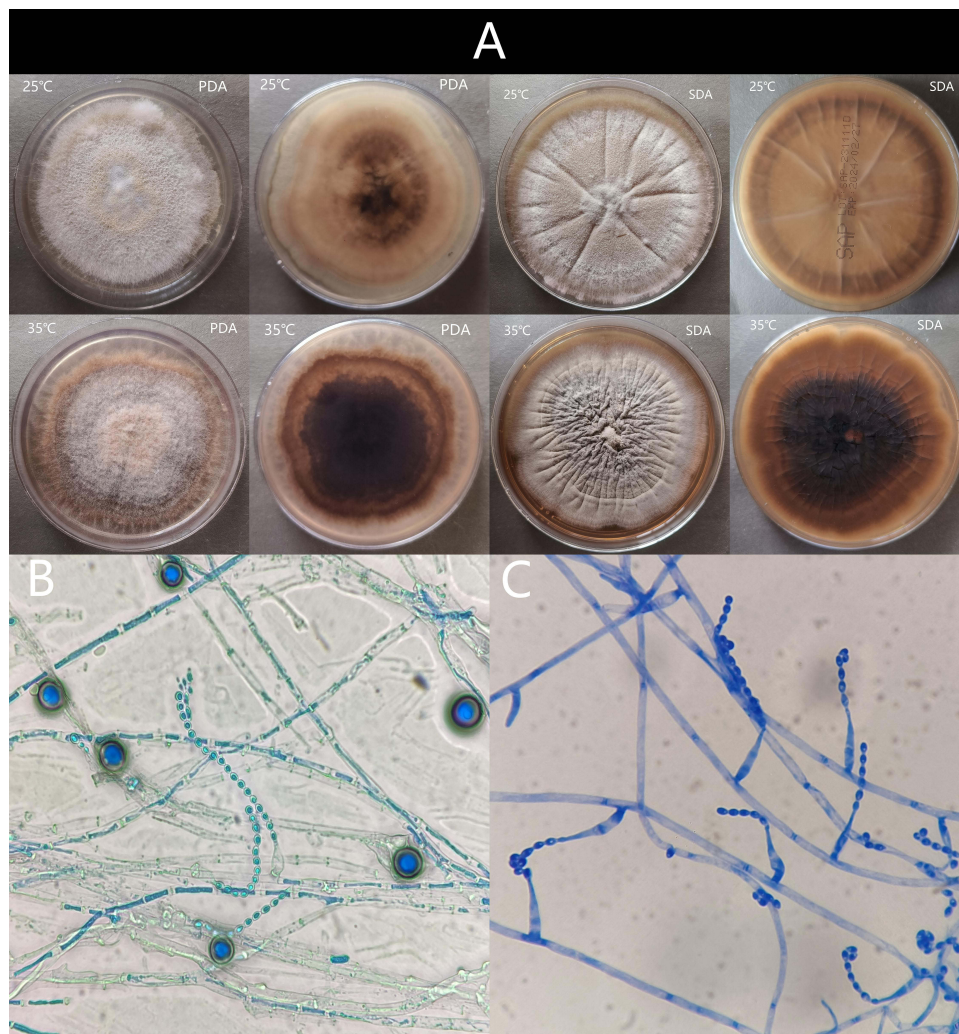


Figure 3 (A) The colonies on PDA and SDA after 14 days incubated at 25°C and 35°C. (B) Micromorphology examination showed two types of conidia: chlamydospores and acromonium-like conidiophores; (C) Acromonium-like conidiophores, which were small ($2.5\text{--}3\text{ }\mu\text{m} \times 1.2\text{--}1.8\text{ }\mu\text{m}$), hyaline, aseptate, smooth, obovoid, with slightly truncate ends, arranged in long divergent chains on the apex of the flask shaped conidiogenous cells (phialides).

conidiogenous cells (phialides) (Figure 3C). The strain was confirmed by Internal transcribed spacer 1 (ITS1) sequencing, and the obtained nucleotide sequence was compared with the nearest sequence at the NCBI GenBank database. The first homology sequence presenting the highest identity was *H. pulvericola* rDNA region (99.81% nucleotide identity).

Antifungal susceptibility testing was performed according to CLSI 38 methods,⁶ and the concentration of suspension was 0.5 Maxwell turbidity. The incubator temperature was 28°C and reading results after 48 h. Determining minimum inhibitory concentrations (MICs) for fluconazole was 8 $\mu\text{g/mL}$, amphotericin B was $>32\text{ }\mu\text{g/mL}$, itraconazole was 0.25 $\mu\text{g/mL}$ and voriconazole was 0.5 $\mu\text{g/mL}$. Minimum effective concentration (MEC) for caspofungin was $>32\text{ }\mu\text{g/mL}$. The results showed that the infectious agent was resistant towards fluconazole, caspofungin and amphotericin B; itraconazole and voriconazole were highly effective against *H. pulvericola*.

The patient was diagnosed with *H. pulvericola* keratitis and treated with oral itraconazole and natamycin eyedrops. After one month treatment, the lesion gradually improved, with the best-corrected visual acuity improving to 0.8.

Discussion

The hyphomycete genus *Humicola* initially identified two species within this genus, which were *H. fuscoatra* (the type species) and *H. grisea*.^{4,7} Over the years, the number of described species in *Humicola* has exceeded 50,^{7,8} but there has

never been a comprehensive revision of the genus, nor a thorough phylogenetic analysis conducted. Most *Humicola* species are typically found in soil, although some have been isolated from compost, decaying plant materials, indoor environments, and even the fur of cats.^{9,10} These species possess potential applications as bio-organic fertilizers or as biological control organisms for plant diseases, including amelioration of arsenic phytotoxicity and use *Humicola* sp to produce novel insecticidal compound.^{11,12} However, it is important to note that certain *Humicola* species have been reported to have detrimental effects on human health. Disease caused by *Humicola* species is rare, with two cases of allergic reactions caused by *Humicola fuscoatra*,^{13,14} a case of *Humicola*-associated peritonitis,⁵ as well as a *Humicola* trauma-related invasive fungal infection.¹⁵

Humicola is commonly recognized as an asexual genus in the *Chaetomiaceae* family.⁴ The microscopic morphology of *Humicola* species may be indistinguishable from other hyphomycete genera with similar thick-walled conidia, such as *Chaetomiaceae*, *Mycothermus*, *Staphylotrichum*, and *Trichocladium*.⁴ Some *Humicola* species can produce two types of conidia: large, dark, globose to subglobose chlamydospores borne singly on vegetative hyphae and small hyalin; and acremonium-like conidiophores, which can distinguish this genus from other similar genus. However, acremonium-like conidiophores only occasionally present and some species do not produce acremonium-like conidiophores.⁴ In our case, *H. pulvericola* colony on PDA and SDA was fluffy, with pale yellow aerial mycelia incubated at 25°C. At 35°C, the colony on PDA was yellow-brown (obverse) and a dark black color (reverse); colony on SDA was grey (obverse), and a dark black color (reverse). *H. pulvericola* colony micromorphology examination showed chlamydospores within a week and produced two types of conidia after 14 days culture: chlamydospores and acremonium-like conidiophores, which can distinguish this genus from other similar genus and members within *Humicola* genus. DNA sequencing is a crucial method for identification *Humicola* species. Two cases of *Humicola fuscoatra* allergic reactions were diagnosed by challenge with antigen inhalation.^{13,14} The culture isolate of *Humicola*-associated peritonitis was identified by morphology and ITS sequencing.⁵ The fungus of trauma-related invasive fungal infection was identified as *Humicola* species by DNA sequencing.¹⁵ In our case, the isolate produced chlamydospores and acremonium-like conidiophores, which were typical features of *Humicola*. Subsequently, ITS1 sequencing confirmed it as *H. pulvericola*. Although characteristics of acremonium-like conidiophores production by *H. pulvericola* have been described, this was only used as a supplement for identification, because culture-based phenotypic methods are insensitive and slow, requiring 2–4 weeks to form typical structures. It is suggested that ITS rDNA sequencing serves as a valuable molecular biological technique for *Humicola* species identification.

Currently, there is a lack of recommended treatment options available for *Humicola* infections. A case of peritoneal dialysis associated peritonitis caused *Humicola* sp. was successfully treated with amphotericin B and voriconazole antifungal therapy, along with multiple operative interventions.⁵ *Humicola* trauma-related invasive infection emphasized aggressive surgical management combined with antifungal drug therapy can optimize patient outcomes.¹⁵ In our case, in vitro drug sensitivity test showed that *H. pulvericola* was resistant towards fluconazole, caspofungin and amphotericin B; itraconazole and voriconazole were highly effective against *H. pulvericola*. According to the MIC values, we successfully treated with oral itraconazole and natamycin eyedrops.

Conclusion

In conclusion, we report first case of fungal keratitis caused by *H. pulvericola* and successfully treated with itraconazole antifungal therapy for one month. Given the taxonomy of the genus *Humicola* is unclear and its similarity with *Chaetomiaceae* family in morphology, it is recommended to use molecular techniques for species identification. Correct delineation of the species and antifungal susceptibility testing will be improving clinical prognosis.

Abbreviations

ITS, Internal transcribed spacer; SDA, Sabouraud glucose agar; PDA, potato dextrose agar; MICs, minimum inhibitory concentrations; MEC, Minimum effective concentration.

Data Sharing Statement

The datasets used and analysed during the current study available from the corresponding author on reasonable request.

Ethics Approval and Patient Consent

Written informed consent was obtained from the patient for the publication of the case details. The study was approved by the medical ethics committee of The People's Hospital of Guangxi Zhuang Autonomous Region.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflict of interest in this work.

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