



Pythiosis: Case report leading to new features in clinical and diagnostic management of this fungal-like infection

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1 Title page

2 **Pythiosis: case report leading to new features in clinical and diagnostic management of**
3 **this fungal-like infection**

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Abstract

A 21-year-old man developed an infectious keratitis after swimming in Spain whilst wearing contact lenses. Mycelial growth from a corneal sample suggested keratomycosis but a drastic worsening of the patient's condition was observed on antifungal drugs. At day 38, the Pan-fungal PCR identified *P. insidiosum*, which is an aquatic organism belonging to the oomycete family. Based on recent literature, our patient was promptly prescribed systemic and local antibiotic regimen and cure was ultimately achieved. In order to facilitate *P. insidiosum* identification in future cases, we have generated the first MALDI-TOF MS reference spectrum for *Pythium insidiosum*. This MALDI-TOF-MS reference spectrum is planned to be deposited on an open-access-platform and should allow immediate identification of the pathogen. Finally, this case report also demonstrates that *P. insidiosum* is emerging outside tropical and subtropical areas. Clinicians and microbiologists should have better knowledge to accurately manage and diagnose this sight-threatening infection.

Keywords: Pythiosis, *Pythium insidiosum*, keratitis, pan-fungal PCR, MALDI-TOF Mass spectrometry

Introducing pythiosis and *Pythium insidiosum*. Pythiosis, also known as ‘swamp cancer’, has only recently been identified in humans. Hosts become infected after contact between a skin injury and zoospore-contaminated water. The form of pythiosis that develops (cutaneous, vascular and ocular) depends on the exogenous inoculation site for this fungus-like pathogen. A case of fatal disseminated pythiosis was recently described in an immunocompromised patient with initial skin involvement (Hilton et al., 2016). Pythiosis is, thus, now considered an emerging human infection (Gaastra et al., 2010; Hilton et al., 2016). The oomycete *P. insidiosum* is the only etiologic agent of pythiosis in mammals. *P. insidiosum* is more closely related to diatoms and algae, but, under the microscope, it appears as a fungus-like mycelium. As pythiosis mimics fungal infections clinically and microbiologically, antifungal agents are widely used for its treatment, resulting in a high rate of treatment failure (Agarwal et al., 2017; Gaastra et al., 2010; Hilton et al., 2016; Krajaejun et al., 2004). Recent *in vitro* and *in vivo* studies have suggested that some antibiotics may be more suitable than antifungal drugs for the treatment of pythiosis (Jesus et al., 2015; Loreto et al., 2014).

We report a clinical case of ocular pythiosis due to the aptly named *P. insidiosum*, which was initially misdiagnosed as keratomycosis. We propose new diagnostic and therapeutic approaches to limit diagnostic errors and the prescription of ineffective treatments.

Ocular pythiosis: the clinician’s view (figure 1- left side). A 21-year-old patient developed symptoms 13 days after water-based activities in the region of Cambrils in Spain whilst wearing his monthly soft hydrophilic lenses. On his return, he presented with a corneal abscess, with significant perilesional infiltrate. The patient was admitted to hospital and initially treated with fortified antibacterial eye drops and anti-amebic treatments (fig. 1). Local degradation was observed, necessitating emergency ultraviolet cross-linking, followed 48 hours later by transfixing keratoplasty (day 12-14). Microbiological analyses of the excised cornea revealed the presence of a mycelium compatible with a filamentous fungus. The

treatment was switched to voriconazole, amphotericin B, hexomedine, PHMB and dexamethasone/neomycin eye drops. After further local degradation, corneo-scleral transplantation was performed (day 21). The dexamethasone drops, were stopped, and treatment with 2% cyclosporine was introduced to prevent graft rejection. Rapidly, whitish bodies were observed in the anterior chamber and treated by five surgical ablation associated with injections of 0.1% voriconazole on days 29, 31 34, 36 and 38. Microbiological examinations of the collections revealed a fungus-like mycelium that could not be identified by conventional methods. A corneal fragment sent for pan-fungal PCR detected the presence of *P. insidiosum* on day 38. Following this identification, and based on the most recent reports concerning its antimicrobial drug susceptibility (He et al., 2016; Jesus et al., 2015; Loreto et al., 2014), antifungals were stopped and antibiotics were prescribed: oral doxycycline and clindamycin, and eye drops containing azithromycin and 2% cyclosporine. Since the initiation of this dual antibiotherapy, no further local degradation has been observed.

Ocular pythiosis: the microbiologist's view (figure 1- right side).

Initial microbiological examinations were performed on material scraped from the cornea on days 0, 3 and 7: PCR for HSV and *Acanthamoeba*, and cultures for the detection of bacteria, fungi and amebae were negative. The patient underwent a first keratoplasty on day 14. Two days later, a small amount of mycelial growth was observed within the corneal fragment on the agar plate, leading to a strong suspicion of keratomycosis. Surprisingly, none of the various subcultures on Sabouraud-chloramphenicol agar plates yielded positive results, whereas subculture in brain-heart-infusion resulted in the rapid growth of a large mycelium at 35°C. All attempts to identify this mycelium on the basis of microscopic morphology or by MALDI-TOF MS failed. Indeed, despite the high quality of the protein spectrum, it could not be attributed to any of the microorganisms present in the Bruker Taxonomy (7815 entries), Bruker Filamentous Fungi (364 MSP), NIH mold (365 profiles) or MSI fungal (> 22,000

spectra) databases. The corneal biopsy specimen and the mycelium were, therefore, sent for pan-fungal PCR diagnosis on day 29. Biomolecular analyses were performed with panfungal primers (ITS1/ITS4 and NL1/NL4) and sequencing results obtained on day 37 unambiguously identified the species present as *P. insidiosum*.

Discussion

Pythium insidiosum has rarely been identified as the causal agent of infectious keratitis, particularly in patients with no history of travel to tropical areas. There is, therefore, no clear algorithm for the management of this mold-like infection. Cryotherapy, absolute alcohol during surgery and antifungal agents administered topically or by injection have all been used, but with poor success rates, and enucleation is frequent (up to 42%) (Agarwal et al., 2017; Lelievre et al., 2015). Adjuvant immunotherapy with *P. insidiosum* antigen injection (PIA) has also been described (He et al., 2016). Recent studies *in vitro* (Loreto et al., 2014) and *in vivo* (Jesus et al., 2015) have highlighted the possible activity of antibiotics against *P. insidiosum*. In particular, macrolides (azithromycin) and tetracyclines (minocycline) have been shown to have synergistic activity *in vitro*, whereas antifungal drugs are ineffective. Oral azithromycin was also found to be the most active treatment *in vivo*. These results strongly suggest that systemic macrolide (+/- tetracycline) treatment should be preferred over antifungal drugs as the first-line treatment for pythiosis. Ramappa *et al.* successfully treated a presumptive case of *Pythium* keratitis with systemic and local regimens of azithromycin and linezolid (Ramappa et al., 2017). Based on these findings, our patient was prescribed this dual antibiotic regimen. However, this treatment was not initiated until 38 days. Similar long delays to the diagnosis of *pythiosis* were reported in all previous cases. The delay was particularly long in this case because, despite the presence of sterile mycelium, successive subcultures were negative. *Pythium* generally grows well on Sabouraud medium, but it did not do so on a Sabouraud supplemented with chloramphenicol. Additional time was

also lost to the various attempts to identify this fungus-like organism by MALDI-TOF-MS, because there is currently no reference spectrum for *P. insidiosum* in the various MSP databases commonly used for microbial identification (Drissner and Freimoser, 2017). Therefore, after confirmation by sequencing, we created a reference MSP on the Microflex mass-spectrometer, according to Bruker's MSP creation protocol (v1.1). This reference spectrum was added to our local database and can be shared on request. It is also planned to be rapidly added to the MSI database (<https://biological-mass-spectrometry-identification.com/msi/welcome>), serving as a free identification platform for MALDI-TOF identification, to facilitate the diagnosis of *Pythium* infections in the future both at our institute and at other healthcare (or veterinary) centers.

Conclusion

We can learn several lessons from this atypical case of keratitis. First, contrary to previous suggestions, *P. insidiosum* does not seem to be limited to tropical and subtropical areas. Even in the absence of travel to the tropics, this etiologic agent should be considered in cases of suspected keratomycosis with water-related exposure, particularly if the infection is resistant to antifungal treatments. Second, our findings suggest that antibiotic-supplemented media should be avoided, whereas Sabouraud agar without antibiotics or yeast extract–peptone–dextrose (YPD) should be used preferentially to ensure accurate diagnostic. Third, although we have little hindsight for the moment, antibiotic treatment seems to be more effective against *Pythium* than antifungal treatments and should be considered as a first-line therapy as soon as the identification is confirmed. Finally, in this case, it was possible to achieve definitive identification of the pathogen by sequencing. However, there is a need to develop rapid unambiguous diagnostic tools to facilitate the identification procedure. For this purpose, we have generated the first MALDI-TOF reference spectrum for *Pythium insidiosum*, which we have made freely available.

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Conflict of interest

No conflict of interest

Ethical Approval

Not required.

References

- Agarwal, S., Iyer, G., Srinivasan, B., Agarwal, M., Kumar, S.P.S., Therese, L.K., 2017. Clinical profile of pythium keratitis: perioperative measures to reduce risk of recurrence. *Br. J. Ophthalmol.* *bjophthalmol-2017-310604*. <https://doi.org/10.1136/bjophthalmol-2017-310604>
- Drissner, D., Freimoser, F.M., 2017. MALDI-TOF mass spectroscopy of yeasts and filamentous fungi for research and diagnostics in the agricultural value chain. *Chem. Biol. Technol. Agric.* *4*, 13. <https://doi.org/10.1186/s40538-017-0095-7>
- Gaastra, W., Lipman, L.J.A., De Cock, A.W.A.M., Exel, T.K., Pegge, R.B.G., Scheurwater, J., Vilela, R., Mendoza, L., 2010. *Pythium insidiosum*: An overview. *Vet. Microbiol.* *146*, 1–16. <https://doi.org/10.1016/j.vetmic.2010.07.019>
- He, H., Liu, H., Chen, X., Wu, J., He, M., Zhong, X., 2016. Diagnosis and Treatment of *Pythium Insidiosum* Corneal Ulcer in a Chinese Child: A Case Report and Literature Review. *Am. J. Case Rep.* *17*, 982–988. <https://doi.org/10.12659/AJCR.901158>
- Hilton, R.E., Tepedino, K., Glenn, C.J., Merkel, K.L., 2016. Swamp cancer: a case of human pythiosis and review of the literature. *Br. J. Dermatol.* *175*, 394–397. <https://doi.org/10.1111/bjd.14520>
- Jesus, F.P.K., Loreto, É.S., Ferreiro, L., Alves, S.H., Driemeier, D., Souza, S.O., França, R.T., Lopes, S.T.A., Pilotto, M.B., Ludwig, A., Azevedo, M.I., Ribeiro, T.C., Tondolo, J.S.M., Santurio, J.M., 2015. In Vitro and In Vivo Antimicrobial Activities of Minocycline in Combination with Azithromycin, Clarithromycin, or Tigecycline against *Pythium insidiosum*. *Antimicrob. Agents Chemother.* *60*, 87–91. <https://doi.org/10.1128/AAC.01480-15>
- Krajaejun, T., Prachartam, R., Wongwaisayawan, S., Rochanawutinon, M., Kunakorn, M., Kunavisarut, S., 2004. Ocular pythiosis: is it under-diagnosed? *Am. J. Ophthalmol.* *137*, 370–372. [https://doi.org/10.1016/S0002-9394\(03\)00908-5](https://doi.org/10.1016/S0002-9394(03)00908-5)

186 Lelievre, L., Borderie, V., Garcia-Hermoso, D., Brignier, A.C., Sterkers, M., Chaumeil, C.,
 187 Lortholary, O., Lanternier, F., 2015. Imported *Pythium insidiosum* Keratitis After a
 188 Swim in Thailand by a Contact Lens-Wearing Traveler. *Am. J. Trop. Med. Hyg.* 92,
 189 270–273. <https://doi.org/10.4269/ajtmh.14-0380>
 190 Loreto, É.S., Tondolo, J.S.M., Pilotto, M.B., Alves, S.H., Santurio, J.M., 2014. New Insights
 191 into the In Vitro Susceptibility of *Pythium insidiosum*. *Antimicrob. Agents*
 192 *Chemother.* 58, 7534–7537. <https://doi.org/10.1128/AAC.02680-13>
 193 Ramappa, M., Nagpal, R., Sharma, S., Chaurasia, S., 2017. Successful Medical Management
 194 of Presumptive *Pythium insidiosum* Keratitis. *Cornea* 36, 511–514.
 195 <https://doi.org/10.1097/ICO.0000000000001162>
 196

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Figure legend

Figure 1: Pythiosis: chronology of events, the clinician's (left) and microbiologist's (right)

viewpoints. D: day; MSP: mass spectrum profile; HSV: herpes simplex virus; PHMB:

polyhexamethylene biguanide. Phaco: phacoemulsification; DMEK: Descemet membrane

endothelial keratoplasty. TK : transfixiant keratoplast

D-13: Probable contamination



Hexamidine & PHMB
Vancomycin & amikacin



Cross linking &
Transfixing keratoplasty



Hexamidine & PHMB
Dexamethason / Neomycin

**Spectacular TK
degradation**



Corneo-scleral graft

Voriconazole IV
Ciclosporin 2%
Amphotericin B
Voriconazole



**Anterior chamber
washout
& voriconazole injections**



Oral doxycycline
Oral clindamycin
Azythromycin
Ciclosporin 2%



**No sign of relaps of the
infection
Deficiency of the corneo-
scleral graft**

Phaco DMEK ? D...

D_{1,3,7}

Corneal scraping

Sterile cultures

■ Bacterial & mycological

Negative PCR

■ *Acanthamoeba* & HSV

D₁₄

Corneal fragments

Mycelium growth

■ **Corneal
biopsy**
■ **BHI media**



Sterile cultures

Negative PCR

■ Bacterial
■ *Acanthamoeba*

D₂₉

Pan-fungal PCR

Sequence in *Sealbac, Jaxima*
Phyllum medusorum isolate CBS 574.05.205 large subunit ribosomal RNA gene, 18S
Accession ID: DQ201223.1 Length: 1074 Number of Reads: 1

Accession	Read	Position	Score	Match	Read
Sealbac	574.05.205	18S	100	100	Sealbac
Sealbac	574.05.205	18S	100	100	Sealbac
Sealbac	574.05.205	18S	100	100	Sealbac
Sealbac	574.05.205	18S	100	100	Sealbac
Sealbac	574.05.205	18S	100	100	Sealbac
Sealbac	574.05.205	18S	100	100	Sealbac
Sealbac	574.05.205	18S	100	100	Sealbac
Sealbac	574.05.205	18S	100	100	Sealbac
Sealbac	574.05.205	18S	100	100	Sealbac
Sealbac	574.05.205	18S	100	100	Sealbac

D38: *P. insidiosum* identification

***P. insidiosum* MSP creation
MALDI-TOF identification**

