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

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ABSTRACT

A 21-year-old man developed 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. On day 38, panfungal PCR identified *Pythium insidiosum*, which is an aquatic organism belonging to the oomycete family. Based on the recent literature, this patient was promptly prescribed a 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 matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) reference spectrum for *P. insidiosum*. It is planned to deposit this MALDI-TOF MS reference spectrum on an open-access platform and this 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.

Introduction

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, or 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 *Pythium insidiosum* is the only etiological 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.

Case report

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 a significant peri-lesional infiltrate. The patient was admitted to hospital and initially treated with fortified antibacterial eye drops and anti-amebic treatments (Figure 1). Local degradation was observed, necessitating emergency ultraviolet cross-linking, followed 48 h later by a penetrating keratoplasty (days 12–14). Microbiological analyses of the excised cornea revealed the presence of a mycelium compatible with a filamentous fungus. Treatment was switched to voriconazole, amphotericin B, hexomedine, polyhexamethylene biguanide (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. These were treated with five surgical ablations 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 panfungal PCR detected the presence of *P. insidiosum* on day 38. Following this

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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. Following the initiation of dual antibiotherapy, no further local degradation was 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 herpes simplex virus 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 on brain–heart infusion medium (BHI) 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 matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (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

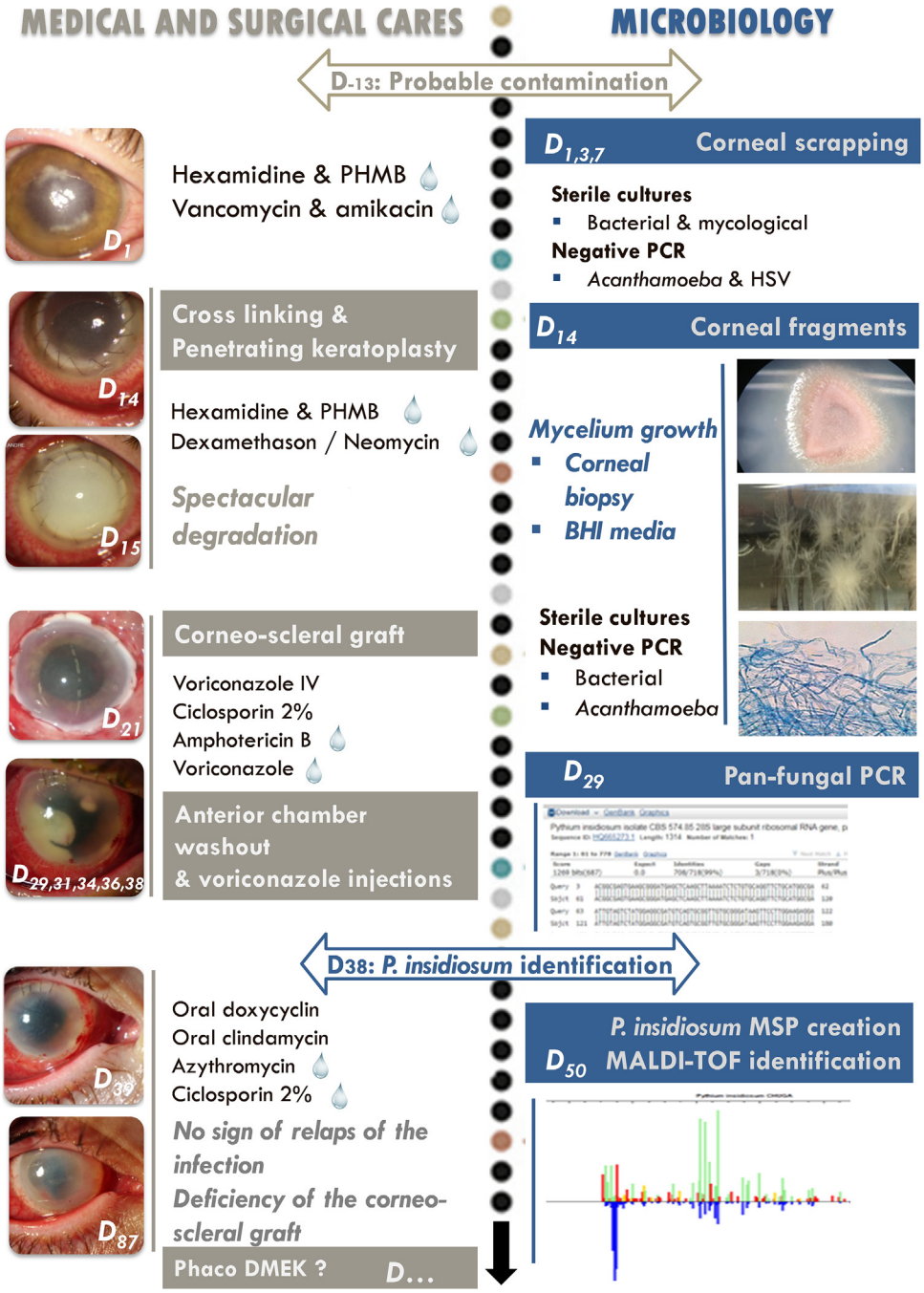

Figure 1. Pythiosis: chronology of events from the clinician's (left) and microbiologist's (right) viewpoints. D, day; MSP, mass spectrum profile; HSV, herpes simplex virus; PHMB, polyhexamethylene biguanide; Phaco, phacoemulsification; DMEK, Descemet membrane endothelial keratoplasty; PK, penetrating keratoplasty.

Taxonomy (7815 entries), Bruker Filamentous Fungi (364 mass spectrum profiles (MSP)), US National Institute of Health (NIH) mold (365 profiles) or MSI (Mass spectrometry Identification) fungal (> 22 000 spectra) databases. The corneal biopsy specimen and the mycelium extracted from BHI were, therefore, sent for panfungal 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 *Pythium insidiosum* (Genbank accession numbers MN148708 and MN148707).

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 in vitro (Loreto et al., 2014) and in vivo (Jesus et al., 2015) studies 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, the case patient was prescribed this dual antibiotic regimen. However, this treatment was not initiated until day 38. Similar long delays to the diagnosis of pythiosis have been 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 Sabouraud medium 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 commercial reference spectrum for *P. insidiosum* in the various MSP databases commonly used for microbial identification (Drissner and Freimoser, 2017), although recent studies have concluded that MALDI-TOF MS is well suited for *P. insidiosum* identification (Krajaejun et al., 2018 and Mani et al., 2019) . Therefore, after confirmation by sequencing, we created a reference MSP on the Microflex mass spectrometer, according to Bruker's MSP creation protocol (v. 1.1). These reference spectra was added to our local database but also to the MSI database platform (https://msi.happy-dev.fr/) to provide a free identification platform for MALDI-TOF identification and facilitate the diagnosis of *Pythium* infections in the future, both at our institute and at other healthcare (or veterinary) centers. Of note, because the 24 added spectra correspond to one strain only, and because of geographical strain diversity, the users of the MSI platform must be aware that it will not provide a 100% sensitivity for *P. insidiosum* identification.

In conclusion, several lessons can be learned 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 etiological agent should be considered in cases of suspected keratomycosis with water-related exposure, particularly if the infection is resistant to antifungal treatments. Second, the study 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 diagnosis. Third, although observations are limited at this time, antibiotic treatment appears to be more effective against *Pythium* than antifungal treatments and should be considered as the first-line therapy as soon as the identification is confirmed. Finally, it was possible to achieve definitive identification of the pathogen by sequencing in this case. However, there is a need to develop rapid unambiguous diagnostic tools to facilitate the identification procedure. For this purpose, we have generated a MALDI-TOF reference spectrum for *Pythium insidiosum,* which we have made freely available on the MSI platform (https://msi.happy-dev.fr/).

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Ethical approval

Not required.

Conflict of interest

No conflict of interest.

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