Medical Mycology Case Reports 42 (2023) 100610

Contents lists available at ScienceDirect

Medical Mycology Case Reports

journal homepage: www.elsevier.com/locate/mmcr

Simultaneous infection with *Fusarium proliferatum* and *Prototheca wickerhamii* localized at different body sites

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ARTICLE INFO

Handling Editor: Dr Adilia Warris

Keywords:
Fusarium proliferatum
Prototheca wickerhamii co-infection
Subcutaneous fungal disease

ABSTRACT

Subcutaneous infections caused by two unusual fungi are rare. Here we report an elderly woman with long-term glucocorticoid use who was infected with *Fusarium proliferatum* on the right dorsum of the hand presenting with a verrucous plaque and *Prototheca wickerhamii* on the left dorsum of the hand presenting with geographic ulcers with erythematous plaques. The diagnosis was made through histopathological examination of skin samples and fungal culture, with identification through molecular examination. She was successfully treated with voriconazole.

1. Introduction

Fusarium proliferatum is a ubiquitous soil inhabitant and plant pathogenic mold belonging to the genus *Fusarium*, but it seldom infects humans [1]. *Prototheca wickerhamii* is the most mainly responsible for human infections which belongs to the genus *Prototheca* [2]. Both are susceptible to infections in immunocompromised populations [1,3]. We herein describe an 81-year-old Chinese woman who has taken oral methylprednisolone tablets for 10 years with *Fusarium proliferatum* and *Prototheca wickerhamii* co-infection that showed great improvement after the systemic use of voriconazole.

2. Case

An 81-year-old woman presented with a verrucous plaque on the right dorsum of the hand for 6 months after being pocked by a moldy wooden frame, and erythematous ulcers on the left dorsum of the hand with no apparent cause for 2 months (day 0). Previous antibiotic treatment had not shown any improvement, and lesions gradually expanded with pain. The patient had a history of hypertension, diabetes, gout, and thrombocytopenia. She has taken oral methylprednisolone tablets for thrombocytopenia at a dose of 8 mg/day for 10 years. Her family history and psycho-social history were unremarkable. Physical examination revealed a warty plaque with a well-defined border on the right dorsum of the hand and irregular edematous erythema, ulceration on the left dorsum of the hand with erythema spreading to the left forearm (Fig. 1).

The morphology of the lesions on both dorsa of the hands was different, verrucous plaque on the right dorsum of the hand and geographic ulcers with erythematous plaques on the left dorsum of the hand, therefore histopathological examination and fungal testing were quickly performed on both dorsa of the hands (day 0).

Histopathological examination of the right dorsum of the hand revealed fungal hyphae in the dermis with Periodic acid–Schiff stain and the histopathological examination of the left dorsum of the hand showed distinct sporangia arranged in a wheel-like pattern in the dermis with hematoxylin and eosin stain (day 7) (Fig. 2 A and D).Fungal cultures of biopsy tissue yielded white and pink fluffy spread colonies on the right dorsum of the hand, and round smooth yeast-like colonies on the left dorsum of the hand (day 7) (Fig. 2 B and E). Direct microscopy of lactophenol cotton blue of the colonies showed hyaline septate fungal hyphae and fusiform conidia of the right dorsum of the hand and mulberry-like sporangia resembling the spokes of a wheel of the left dorsum of the hand (day 7) (Fig. 2C and F). The isolate from the right dorsum of hand was identified by sequencing the ITS and RBP2 regions. Both sequences

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https://doi.org/10.1016/j.mmcr.2023.100610
Received 28 July 2023; Received in revised form 14 September 2023; Accepted 20 September 2023
Available online 21 September 2023

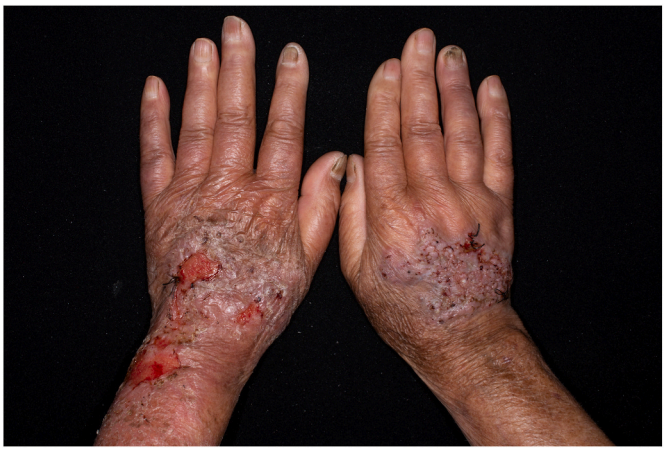

Fig. 1. Clinical manifestations. Edematous erythema and ulceration with irregular boundaries on the left dorsum of the hand. Well-defined verrucous plaque with multiple black dots on the right dorsum of the hand.

indicated that the isolate got high similarity to *Fusarium proliferatum* (100% similar to *Fusarium proliferatum* strain BL4 in ITS region, GenBank number MT466521.1 and 99% similar to *Fusarium proliferatum* strain ET1 in RBP2 region, GenBank number XM_031231602.1); The identification of the culture from left dorsum of hand was achieved by sequencing the cytb region and the result showed 100% similarity with *Prototheca wickerhamii* strain ATCC MYA4772 cytochrome *b* (cytb) gene (GenBank number OP748362.1) (day 10). In vitro antifungal susceptibility testing was performed by broth microdilution according to the Clinical and Laboratory Standards Institute(CLSI) M38-A3 and M27-A3. Minimum inhibitory concentrations (MICs)values were determined for a variety of antifungals (Table 1) (day 10).

The patient was diagnosed with subcutaneous fungal disease caused by *Fusarium proliferatum* and *Prototheca wickerhamii* co-infection based on clinical manifestations, histopathological and laboratory findings. According to antifungal susceptibility testing, the patient was treated with oral voriconazole (400 mg/day) (day 11) for four weeks.

Table 1
Antifungal susceptibility testing against various antifungal agents for *Fusarium proliferatum* and *Prototheca wickerhamii*. Note: There are no universally accepted guidelines applicable to *Prototheca* species, the determination of the MIC was performed strictly following the Clinical and Laboratory Standards Institute (CLSI) protocol (M27- A3) for the drug susceptibility testing of yeasts.

Antifungal Agent	*Fusarium proliferatum* MICs (μg/ml)	*Prototheca wickerhamii* MICs (μg/ml)
Amphotericin B	4	0.25
Anidulafungin	> 8	> 8
Caspofungin	> 8	> 8
Voriconazole	2	1
Posaconazole	2	1
Itraconazole	> 16	1
Fluconazole	> 256	> 256
Terbinafine	> 16	–
Flucytosine	> 64	> 64

Transaminase levels was performed every 7 days, with no laboratory and clinical adverse events. After clinical cure had been achieved, no recurrence of the disease was detected over a 5-month follow-up visit, leaving scarring and hyperpigmentation (Fig. 3).

3. Discussion

There are some reports about the infection of *Fusarium proliferatum* or *Prototheca wickerhamii* in humans [3] [–] [6], but the study presents the first case of both fungi co-infection in a patient as we know. *Fusarium* is a filamentous fungus widely found on plants and soil. The genus currently contains over 100 species. The most frequent human *Fusarium* isolates are (in decreasing order) *F.solani*, *F.oxysporum*, and *F.proliferatum* [1]. The clinical manifestations of *Fusarium* infections depend on the host immune system condition. In humans with normal immune systems, *Fusarium* spp. often cause onychomycosis and keratitis. While immunocompromised humans, especially neutropenic patients, *Fusarium* spp. can cause cellulitis, sinusitis, pneumonia, arthritis, osteomyelitis, and disseminated disease with characteristic skin lesions and a high mortality rate [7]. For mucocutaneous manifestations of the infection, the most common are umbilicated papules, pustules, cellulitis, and

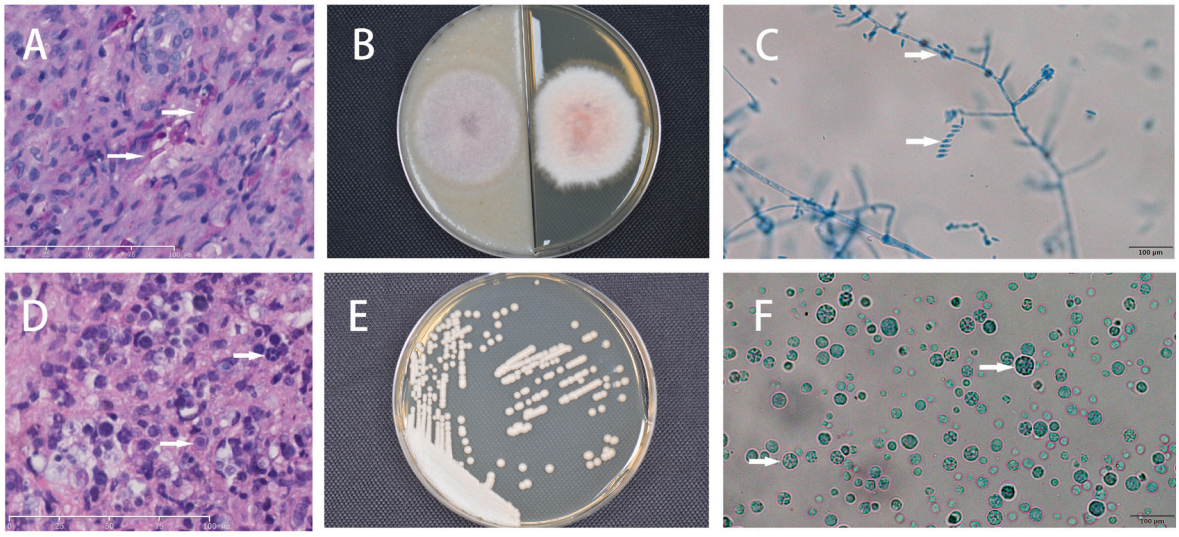

Fig. 2. Histopathology and mycological examinations. Histopathological examination revealed fragmented septate fungal hyphae in the dermis of the right dorsum of the hand (A Periodic acid–Schiff stain, 400x) and spherical sporangia containing multiple endospores in the dermis of the left dorsum of the hand (D hematoxylin and eosin stain, 400x); White and pink fluffy spread colonies on Oatmeal agar and Sabouraud dextrose agar of the right dorsum of the hand (B) and round smooth yeast-like colonies on Sabouraud dextrose agar of the left dorsum of the hand (E); Fungal structures are more visible by direct microscopy, branched, segregated mycelium and fusiform conidia on the right dorsum of the hand (C lactophenol cotton blue stain, 100x) and more evident wheel-like pattern intracellular spores on the left dorsum of the hand (F lactophenol cotton blue stain, 100x). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

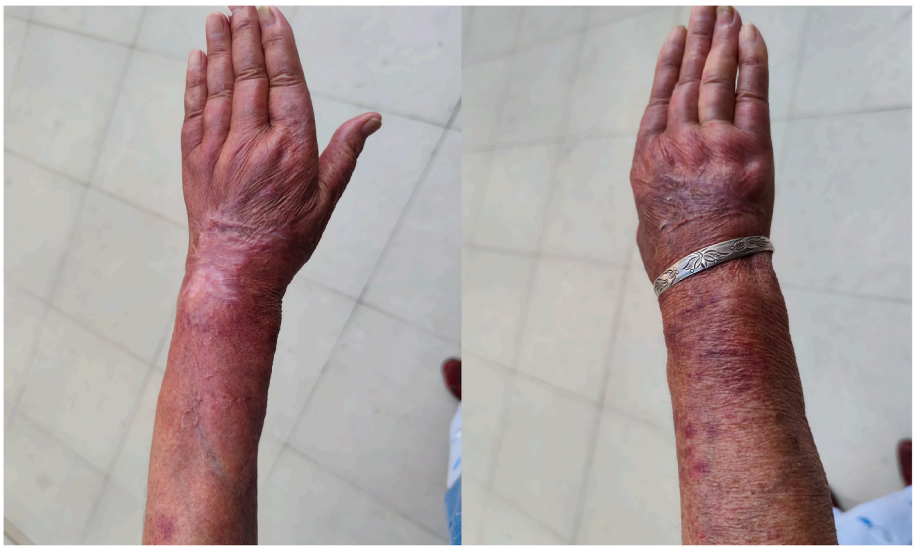

Fig. 3. Clinical image after treatment. After four weeks of voriconazole treatment, scars and hyperpigmentation have form on the right and left dorsa of the hands.

subcutaneous nodules [8]. In most localized deep infections, a history of direct trauma is an important clue for infection [9]. In the case the patient also had a traumatic trigger, this could be the portal of infection. The diagnosis of *Fusarium* is established by detecting fungal elements, which can be performed by conventional microbiological methods or molecular techniques [10]. In this case, the diagnosis of *Fusarium proliferatum* was carried out by direct visualization of fungal hyphae in tissue biopsy, positive culture, leading to administration of the antifungal susceptibility testing and molecular identification.

So far, the genus *Prototheca* is composed of at least eight species, like *P wickerhamii*, *P zopfii*, *P blaschkeae*, *P cutis*, *P miyajii*, *P stagnora*, *P ulmea*, and *P tumulicola*. *Prototheca wickerhamii* is the most mainly responsible for human infections [11]. The occurrence of protothecosis can be local or disseminated and acute or chronic, with the latter being more common. Protothecosis has been classified in three clinical forms, cutaneous lesions, olecranon bursitis, and disseminated or systemic infections [12]. Cutaneous lesions often expose to contaminated water or with a traumatic trigger, usually appear as an erythematous plaque that can be ulcerative with crusting and purulent discharge [13]. However, in the present case, the patient's left dorsum of the hand has no apparent trauma history. We infer that infection of *Prototheca wickerhamii* may be due to sewage exposure. The diagnosis of *Prototheca* usually depends on morphological identification of the organisms in wet slide preparations of cultures and/or direct identification in tissue specimens [14]. *Prototheca* species reveal yeast-like colonies on Sabouraud dextrose agar but differ from fungi as they lack glucosamine in their cell walls [2]. Microscopic examination of the organism in culture reveals the same structures as those observed in tissue (spherical sporangia containing multiple endospores). Intracellular spores are characteristic for *Prototheca* species, yet the number and size are influenced by the culture medium and incubation time [12,14].

Fusarium species may be resistant to most antifungals, however, various species may have different susceptibility patterns. In general, most *Fusarium* isolates are sensitive to amphotericin B rather than azoles (itraconazole, voriconazole and posaconazole) [15]. Candidate antifungal drugs for *Prototheca* infection include amphotericin B and azoles, if possible, antifungal susceptibility testing is necessary [16]. In this case, antifungal susceptibility testing indicated that amphotericin B, voriconazole and posaconazole were sensitive to *Fusarium proliferatum* and *Prototheca wickerhamii*, considering that the patient was older and had many underlying diseases, the tolerance to amphotericin B might be poor, therefore we chose voriconazole for the treatment. Fortunately, the patient's skin lesions achieved a significant improvement after taking voriconazole (400 mg/day) for 4 weeks, and no new lesions were seen.

In the present case, the patient had an exposed skin lesion on the right dorsum of her handcaused by a moldy frame which may have resulted in the *Fusarium proliferatum* infection. However, there was no obvious trauma history of the left dorsum of her hand. We suppose the infection of *Prototheca wickerhamii* may have been acquired following minor skin damage and exposure to sewage exposure. The prolonged use of oral methylprednisolone most likely has made her more susceptible to deep skin infection.

Declaration of competing interest

There are none.

Ethical Form

Please note that this journal requires full disclosure of all sources of funding and potential conflicts of interest. The journal also requires a declaration that the author(s) have obtained written and signed consent to publish the case report from the patient or legal guardian(s).

The statements on funding, conflict of interest and consent need to be submitted via our Ethical Form that can be downloaded from the submission site www.ees.elsevier.com/mmcr. **Please note that your manuscript will not be considered for publication until the signed Ethical Form has been received.**

Acknowledgements

We thank the patient for participating in this study and for her consent to publication. This work was funded by the National Science and Technology Infrastructure of China (National Pathogen Resource, Center NPRC-32).

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