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Case Report

Phaeohyphomycosis due to *Exophiala xenobiotica* as a cause of fungal arthritis in an HIV-infected patient

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Black yeasts including *Exophiala* species are increasingly recognized as agents of human disease. In recent years, progress in molecular phylogeny and taxonomy of the genus *Exophiala* has led to the description of numerous novel species. The 'classical' but highly variable species *Exophiala jeanselmei* was split into a number of morphological siblings, which, however, were phylogenetically and clinically remote from each other. *E. jeanselmei* was restricted to an uncommon species causing subcutaneous infections. Hence only limited information is available on the segregants, among which is *E. xenobiotica*. We describe a case of an HIV-patient presenting with fungal arthritis and subcutaneous nodules caused by the latter species, which was identified by means of phenotypic and molecular methods.

Keywords *Exophiala xenobiotica*, arthritis, black yeast, immune reconstitution inflammatory syndrome, molecular identification, phaeohyphomycosis

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Introduction

Exophiala species are widely distributed in toxic or nutrient-poor habitats of anthropogenic out- and indoor origin, such as industrially polluted soils, bathing facilities or dishwashers [1–4]. Some species are regular colonizers of the respiratory tracts of patients with cystic fibrosis [5,6], while other species are invasive and cause infections ranging from cutaneous to systemic, life-threatening diseases, sometimes with severe mutilation [3,7]. With recent advances in molecular phylogeny, the taxonomy of potentially pathogenic *Exophiala* species has been considerably reevaluated. In the case of the 'classical' species *Exophiala jeanselmei*, this has led to the description of cryptic species, *E. xenobiotica* and *E. oligosperma*. The segregants are much more frequently encountered in the clinical setting than *E. jeanselmei sensu stricto*, a species consistently causing subcutaneous infections [8]. A recent study highlighted that the newly recognized species accounted for more than one third of the clinical black yeast isolates in the USA [9]. Most infections previously attributed to *E. jeanselmei sensu lato* are actually caused by other species, and as a result only limited data are available on the prevalence and pathogenic potential of the siblings [10–12]. Documented case reports are therefore urgently needed. Here, we report on an HIV-patient presenting with arthritis and subcutaneous nodules due to *Exophiala xenobiotica*. Clinical features, prognosis, as well as mycological characteristics of *E. xenobiotica* infections are discussed.

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Case report

A 53-year-old male patient living in France since 1980 (originally from Guinea-Bissau), who was responsible for washing the tanks of oil tankers, was admitted to our hospital because of multiple subcutaneous nodules. Nine months earlier he was given antiretroviral therapy (HAART) following a diagnosis of HIV-1 infection (nadir CD4 + cell count = 11 cells/mm^3, viral load = 3.5 log/ml). Despite significant immunodeficiency, the patient had no history of opportunistic infections or other AIDS-defining illnesses. Under HAART, the HIV viral load decreased rapidly to remain undetectable (below 20 copies/ml), but unfortunately his CD4 + cell count re-established very slowly (47 cells/mm^3).

At physical examination, the patient presented with a few black subcutaneous and sub-aponeurotic tumefactions on the arm and forearm. The largest of these was located on the right wrist and a left-knee hygroma was also noted (Fig. 1). The patient had no fever and could not recall any recent history of trauma. Surgery to excise the tumefaction and hygroma was performed and cutaneous and synovial biopsy specimens were sent to the laboratory for histopathology and culture. Examination of the sub-aponeurotic tumefaction and hygroma synovial tissue samples revealed numerous and well-organized epithelioid granulomas with necrosis containing septate hyphal fragments with ovoid fungal elements, along with multinucleate giant cells (Fig. 2). Similar histopathology was found in the wrist tissue sample. Notably, the presence of exogenous material was noted in the latter sample (Fig. 3). In agreement with biopsy findings, direct microscopic examination of the samples after Gomori-Grocott staining revealed several fungal elements. After a few days, fungal cultures on Sabouraud's dextrose agar of all samples yielded several restricted, dark-olivaceous colonies of yeast-like appearance, leading to the diagnosis of a phaeohyphomycosis. Fungal colonies grew very slowly at 22°C and 30°C, changing from yeast-like to velvety in a later stage of development (Fig. 4). Microscopic examination revealed black, septate hyphae and one-celled conidia suggestive of an *Exophiala* species.

Molecular identification of the isolate was performed. Briefly, fungal DNA was extracted from culture using Macherey-Nagel Nucleospin Tissue kit (Macherey-Nagel, Düren, Germany) and amplification of the ITS rDNA was achieved using ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers on an Applied Biosystems 9700 thermocycler (Applied Biosystems, Foster City, CA, USA). Reaction mixtures contained 0.5 µmol/l of each primer, 10 µl of 5 × buffer, 2 mmol/l of $MgCl_2$, 0.2 mmol/l of each deoxyribonucleoside triphosphate, 0.03 U of GoTaq Flexi DNA polymerase (Promega, Madison, WI, USA), and sterile water up to a final volume of 50 µl. Amplification parameters were as follows: initial denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 45 sec and elongation at 72°C for 1 min. A final extension step at 72°C for 7 min was included at the end of the amplification. PCR products were purified and sequencing was performed using a BigDye terminator

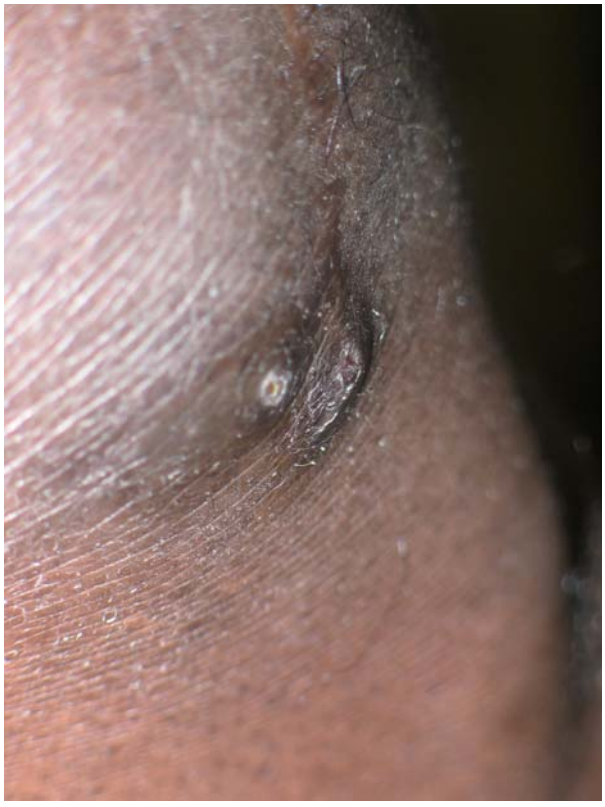

Fig. 1 Tumefactions (hygroma) and lesions located on the left-knee.

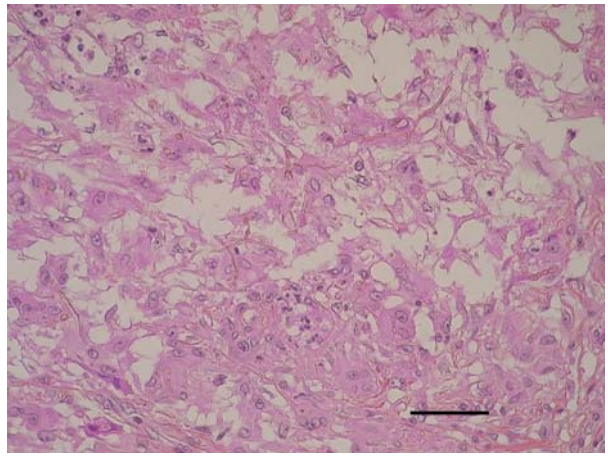

Fig. 2 Histopathological examination showing several, slightly melanized and septate hyphae after hemalun-phloxin-saffron (scale bar = 50 µm).

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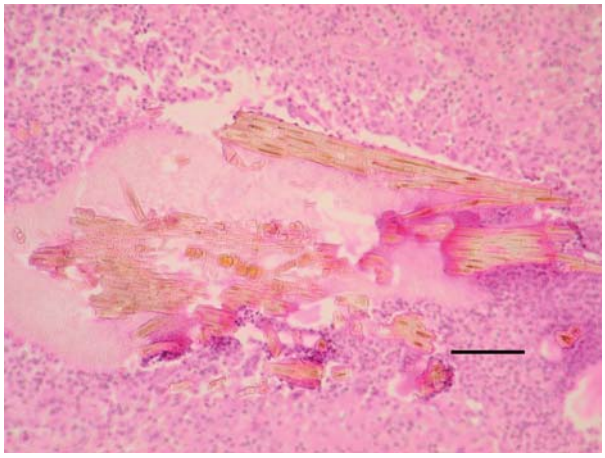

Fig. 3 Histopathological examination of the wrist tissue sample revealing an intense granulomatous and inflammatory reaction surrounding an exogenous material after Hematoxylin Eosin Safran staining (scale bar = 100 μm).

Fig. 4 Macroscopic feature of the *Exophiala xenobiotica* **i**solate grown on Sabouraud's dextrose agar plate after 12 days at 30°C.

sequencing kit on an ABI PrismR 3130 genetic analyzer (Applied Biosystems). Nucleotide sequences were analyzed using Seqscape software (Applied Biosystems). Comparison of the nucleotide sequences of our isolate (577 bp) with GenBank database revealed a 97.3% similarity with that of *E. xenobiotica*, strain CBS 117641 (GenBank DQ182591). DNA amplification and sequencing of the D1/D2 region (576 bp) of the 28S rDNA using NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) primers (same conditions to those described for ITS) confirmed a close genetic proximity with *E. xenobiotica* (98.8% similarity with CBS 115831, GenBank FJ358246). Nucleotide sequences of our isolate (ITS rDNA and D1/D2) have been deposited in GenBank under accession numbers JN621898 and JN621899.

The strain was subsequently sent to the French National Reference Center for Mycoses and Antifungals (CNRMA, Paris, France) for antifungal susceptibility testing according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standardized methodology with slight modifications [13]. The *E. xenobiotica* isolate exhibited low minimal inhibitory concentrations (MICs) to amphotericin B (0.25 μg/ml), voriconazole (0.03 μg/ml), itraconazole and posaconazole (≤ 0.015 μg/ml), but moderately high MIC against caspofungin (1 μg/ml). The *E. xenobiotica* isolate has been deposited at the Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands) with accession number CBS 128473.

Despite absence of any antifungal therapy, the patient's condition improved and six months after the initial diagnosis of phaeohyphomycosis due to *E. xenobiotica*, no recurrence was noted.

Discussion

Exophiala species and their relatives are opportunistic black yeasts, widely distributed in the domestic environment in moist, hot, or extreme (micro) habitats, such as Turkish steam baths [4], dishwashers [1], industrial biofilters [14], or environments enriched by toxic hydrocarbons [14–16]. They are of medical interest because of their ability to provoke a wide spectrum of diseases including life-threatening infections [3,7]. At present, 16 distinct *Exophiala* species have been described from human infections, a large share of these belonging to the *E. spinifera* clade [3,9]. In recent years, an important step in the taxonomy of the genus has been made with the reevaluation of *E. jeanselmei*, which previously was a genetically highly heterogeneous species. Among the newly described molecular siblings was *Exophiala xenobiotica* [14]. However, the pathogenic potential of the novel species is still poorly known and possibly underestimated, due to a low number of studies and case reports where unequivocal species identification was performed [9,12]. Here, we describe the case of an HIV-infected patient presenting with knee arthrosynovitis and subcutaneous nodules, proven to be due to *E. xenobiotica* as identified by means of phenotypic and molecular methods.

From literature data it was apparent that *E. xenobiotica* has been repeatedly isolated from habitats rich in monoaromatic hydrocarbons [14,15]. Interestingly, the patient's anamnesis of job and activities revealed that during his work in France over several years he had regularly been exposed to aromatic compounds, as he was responsible for washing the tanks of oil tankers. The presence of foreign material in one of the biopsy tissue specimens of the wrist led us to hypothesize that the original source of infection of our patient was through inoculation with

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the contents of the tank. Rather than multiple traumata involving both right arm and left knee, hematogenous dissemination, as described in a previous case involving *E. xenobiotica*, would be the most probable hypothesis to explain the recovery of *Exophiala* at distinct non-contiguous sites [9].

It is noteworthy that our patient's conditions improved greatly without application of any antifungal therapy. We hypothesize that the introduction of antiretroviral therapy, nine months before, has been responsible for an immune reconstitution inflammatory syndrome (IRIS) unmasking a latent *E. xenobiotica* infection. The long delay in cure might be explained by the very slow increase of CD4 cell counts during this period. Cases of IRIS revealing invasive fungal infections have been reported in cryptococcosis [17] and histoplasmosis [18], but to the best of our knowledge, have never been described with black yeasts. The great majority of *Exophiala* infections are in otherwise healthy individuals or in patients with innate immune disorders, AIDS-related cases being highly exceptional [19].

In a recent study, *E. xenobiotica* was most frequently associated with mild to moderate superficial or cutaneous infections, occasionally occurring subcutaneously [9]. A single systemic case was reported [14], but in the main, *E. xenobiotica* is less virulent than other *Exophiala* species. For example, *E. dermatitidis* and *E. spinifera* are more frequently involved in potentially life-threatening infections [20], and also than *E. jeanselmei*, agent of subcutaneous mycoses [8]. Convincing evidence that significant differences in virulence exist among *Exophiala* species has recently been provided using a murine model [21]. In this study, mice infected with *E. xenobiotica* had the lowest mortality rate compared to those infected with either *E. dermatitidis* or *E. oligosperma*. Identification of *Exophiala* down to the species level is therefore of medical interest. Recently, a synoptic diagram supporting phenotypic identification of human-associated black yeasts has been proposed [22]. However, not all species are phenotypically distinguishable as distinct species may exhibit similar physiological and morphological characteristics. Species identification within *Exophiala* remains a challenge for non-reference laboratories as ITS sequencing is the current standard. Thanks to the widespread development of molecular methods for fungal identification and taxonomy, ITS rDNA barcoding, in conjunction with conventional methods provides reliable identification of all species of the genus [22]. Here, unequivocal identification of *E. xenobiotica* was reached by means of sequence analysis of ITS and 28S rDNA regions.

At present, no consensus regarding the optimal antifungal therapy of *Exophiala* infections have emerged, but azole antifungals, especially itraconazole and posaconazole have been associated with good clinical outcome [23,24]. Murine models of disseminated phaeohyphomycosis caused by *Exophiala* species have confirmed the efficacy of azole drugs, posaconazole being the most effective against the neurotropic species *E. dermatitidis* [21]. Data on clinical management of *E. xenobiotica* infections are scarce. In one case report, cure was noted after surgery and oral itraconazole therapy in a patient with a non-Hodgkin lymphoma [12,14]. Until now, there is no clear data suggesting that antifungal susceptibilities would differ between species but *in vitro* antifungal susceptibility data remains scarce, especially for *E. xenobiotica* [9,25]. Apart from *E. attenuata* that exhibited resistance to amphotericin B [9], all species have low MICs to itraconazole, voriconazole, posaconazole and amphotericin B [26,27]. Nevertheless, due to the absence of well-defined breakpoints for this genus, correlation with clinical success is still difficult [25].

We hope that further studies with unequivocal identification of *Exophiala* clinical isolates will be performed. This is necessary for a better understanding of the pathogenic potential of black yeasts, as well as for improvement of diagnostics and clinical management. The present report also underlines the utility of molecular identification in the clinical mycology laboratory, in conjunction with conventional methods, to help in the state-of-the-art identification of clinically relevant fungi.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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