**Disseminated fungal infection in an HIV-infected patient due to *Aureobasidium pullulans***

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In South Africa, novel fungal pathogens have emerged as an important cause of disease in HIV-infected individuals. The clinical presentation is characteristically prolonged, insidious and the diagnosis elusive, often being made post mortem. We report an HIV-infected individual with a systemic mycosis due to *Aureobasidium pullulans*, a ubiquitous dematiaceous mould which has not been described in HIV-infected individuals.

**Keywords:** *Aureobasidium pullulans*, disseminated fungal infection, HIV, immune suppression, septic arthritis, systemic mycosis

**Introduction**

Invasive opportunistic systemic mycoses are common in immunosuppressed individuals, with mortality in HIV-infected individuals reaching 48%. The high mortality is in part due to the difficulty in making the diagnosis: fungal culture is slow, hence positive cultures may be missed by the treating clinician. Additionally, the clinical manifestations of disseminated fungal infections are protean, with deep fungal infections in some HIV-infected patients misdiagnosed as tuberculosis, varicella, Kaposi’s sarcoma and papular pruritic eruption of HIV. Thus, the diagnosis is often delayed, and made after death in up to 24% of cases.

The recent description of a novel systemic mycosis, caused by *Emmonsia spp.*, in HIV-infected individuals highlights the need for heightened surveillance in this high-risk population. We describe an HIV-infected patient with a disseminated fungal infection caused by *Aureobasidium pullulans*, a ubiquitous dematiaceous mould that is not known to cause disease in HIV-infected individuals.

**Case report**

In August 2014, a 28-year-old HIV-infected female presented to Chris Hani Baragwanath Academic Hospital (CHBAH) with a four-month history of an insidious, asymmetrical oligoarthritis involving her ankles, knees and left elbow (Figure 1A). She had been previously admitted to CHBAH in June 2013 when she had been diagnosed with cryptococcal meningitis, based on cerebrospinal fluid culture. She was successfully treated with amphotericin B and fluconazole and discharged on fluconazole secondary prophylaxis. During the same admission, she had been diagnosed with HIV-infection, with a baseline CD4+ cell count of 15 cells/mm³. She had initiated antiretroviral treatment (tenofovir/lamivudine/efavirenz) after discharge in July 2013. Five months after initiation of antifungal treatment, her fluconazole prophylaxis was stopped prematurely. She had a period of good health, until April 2014, when her joints became warm, swollen and tender, ultimately resulting in her current presentation. She had an associated rash that was well-circumscribed with scaling plaques on her face, trunk and thighs (Figure 1B). The remainder of her clinical examination was non-contributory. Her chest radiograph showed a small left hilar consolidation, while limb radiographs revealed lytic lesions suggestive of osteomyelitis in her right talus and tibia (Figure 1C and D, respectively). Her current CD4+ cell count was 168 cells/mm³ and her HIV-1 viral load was less than 20 copies/ml. A clinical diagnosis of septic arthritis was made and she was initiated on ceftriaxone and clavulanic, treating empirically for *Neisseria gonorrhoeae* and *Staphylococcus aureus* whilst awaiting microbial confirmation from blood and joint aspirate cultures. An orthopaedic consult was obtained, and an arthrotomy of her right ankle and knee was performed. Tissue histology revealed necrotising granulomatous inflammation with unspecified fungal elements (Figure 1E). A fungus was cultured from blood, synovial fluid and a tissue specimen. This fungus was initially identified as *C. neoformans* (AuxaColor, BioRad, USA), leading to a diagnosis of disseminated cryptococcosis. Her therapy was altered to amphotericin B and fluconazole. A lumbar puncture revealed normal, sterile cerebrospinal fluid (CSF) while her serum and CSF cryptococcal latex antigen test (CrAg) (ImmunoMycologics, USA) was negative. A repeat serum CrAg test two weeks later was positive (titre 2048). The discordant serological results led to questioning of the diagnosis of relapsed disseminated cryptococcosis. Microscopic review of the isolates revealed atypical fungal morphologies not fully in keeping with *C. neoformans*. Two isolates, cultured from blood and synovial fluid, were submitted to a reference laboratory for phenotypic and molecular identification. The synovial fluid isolate was identified as *A. pullulans* phenotypically (urease-positive cream-coloured yeast-like colonies that darkened with age) and genotypically by sequencing of the internal transcribed spacer (ITS) region of the fungal ribosomal gene. The second isolate was...
contaminated and could not be identified further. The patient was continued on amphotericin B and fluconazole for 22 days, resulting in full resolution of the skin lesions and arthritis. She was discharged, in good health and on antiretroviral treatment with fluconazole prophylaxis.

Microbiology
The admission aerobic blood culture was cultivated at 37 °C, and was positive after 32 h, with the initial Gram stain showing no bacteria or yeasts. On subculture, no growth was noted after 24 h; but, after 48 h, small white, dry (almost chalky) colonies grew on both the blood and chocolate agar plates. After approximately 14 days, the colonies darkened and became black (Figure 1F). A Gram stain from this culture revealed ellipsoidal yeast-like cells of varying size, with budding and hyphal structures, not in keeping with typical C. neoformans morphology. These cultures were initially identified as C. neoformans (AuxaColor, BioRad, USA). The AuxaColor kit identifies a limited number of commonly encountered yeast species based on biochemical and morphological criteria. Uncommon fungal isolates with similar morphology and biochemical properties may generate a profile similar to a species in the database of the AuxaColor identification kit, as we believe occurred between C. neoformans and A. pullulans. Upon sub-culture, the aerobic blood culture was found to be contaminated, and could not be identified further. Culture of the synovial fluid from the patient’s right ankle revealed identical colony morphologies and Gram stain characteristics to those from the blood culture. Again, initial culture identification revealed C. neoformans (AuxaColor, BioRad, USA). However, upon sequencing of the ITS region of the multi-copy ribosomal gene of this isolate, A. pullulans was identified (99% sequence homology to A. pullulans, GenBank accession EF595769.1).

Discussion
Invasive fungal infection is rare, and is associated with immunosuppression and medical instrumentation. Worldwide, common human fungal pathogens include Aspergillus and Candida, while other fungi such as Histoplasma, Blastomyces, Coccioidiel, Cryptococcus, and Sporothrix are rarer causes. These fungi can be difficult to diagnose. In South Africa, misidentification of invasive fungal pathogens is common in HIV-infected patients, while profound immunosuppression from HIV-infection allows novel fungal pathogens to cause disseminated disease. Strong clinical and laboratory interrogation is, therefore, warranted when HIV-associated fungal infections are suspected. In our case, C. neoformans was initially cultured but, upon more robust interrogation, A. pullulans was identified. This is possible as A. pullulans is morphologically similar to C. neoformans on early culture, but A. pullulans colonies tend to darken after one to two weeks. Molecular techniques are often required to make a definitive diagnosis.

A. pullulans fungemia has been previously described in patients with either malignancy, recent major surgery, indwelling catheters or bone marrow transplantation. Care must be taken in making the diagnosis of A. pullulans infection, as it has been a contaminant in previous studies. As the phenotypic characteristics of the fungus cultured from all sites in this case were consistent with A. pullulans and sequencing of the ITS region identified A. pullulans, we favour a diagnosis of disseminated A. pullulans infection. The lack of molecular confirmation of the second A. pullulans isolate from our patient, combined with the discordant serum CrAg test results make a definitive diagnosis hard to achieve. The serum CrAg test has been shown to be falsely reactive in the presence of streptococcal antigenemia, and at the time of the second CrAg test, our patient was diagnosed with an infection of viridans group Streptococcus based on three positive blood cultures. We postulate that this may have caused the high positive serum CrAg test result that was discordant with the first. Unfortunately, no further specimens were available to confirm the diagnosis and the patient has not followed up for a repeat serum CrAg test.

The optimum treatment of A. pullulans has not been standardised, and previous reports have suggested amphotericin B, flucytosine and fluconazole to be efficacious. Additionally, amphotericin B has been shown to achieve adequate sterilising concentrations in the synovial fluid. For these reasons, we chose to treat our patient with amphotericin B and fluconazole in a manner that mirrors the guidelines established for C. neoformans. This regimen achieved a complete clinical response in our patient, and should be considered when confronted with HIV-infected patients with disseminated fungal infections, especially A. pullulans.

Conclusion
A. pullulans is a potential opportunistic systemic mycosis in profoundly immunosuppressed HIV-infected individuals. The clinical manifestations of this and other systemic mycoses are protean and often misdiagnosed, leading to high morbidity and often mortality. An immune reconstitution inflammatory syndrome is also likely to ensue if highly active antiretroviral...
treatment is initiated, due to the high pathogen load, and profound immune suppression. Therefore, high clinical suspicion and aggressive pursuit of a diagnosis of disseminated mycosis in all World Health Organisation stage four HIV-infected individuals is warranted.

Declaration – The authors declare that this is their own original work. No conflicts of interest are reported. Informed consent was obtained, and ethical clearance was obtained from the University of the Witwatersrand (Certificate M141203).

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