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Death by Edible Mushroom: First Report of *Volvariella volvacea* as an Etiologic Agent of Invasive Disease in a Patient following Double Umbilical Cord Blood Transplantation▽

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We describe a case of invasive fungal infection caused by *Volvariella volvacea* following double umbilical cord blood transplantation (UCBT). Although infections caused by several mushroom species have been documented, we believe this to be the first published report of invasive infection with *Volvariella volvacea*, an edible mushroom belonging to *Agaricales*.

CASE REPORT

A 41-year-old Indian female living in Barbados was diagnosed with stage IV nodular sclerosing Hodgkin's lymphoma in July 2007. She was initially treated with 6 cycles of Adriamycin (doxorubicin), bleomycin, vinblastine, and dacarbazine (ABVD) chemotherapy, with only a partial response. Therefore, she required second-line treatment with 2 cycles of ifosfamide, carboplatin, and etoposide (ICE) chemotherapy followed by autologous stem cell transplantation (ASCT). She relapsed 5 months following ASCT (August 2008) and received salvage chemotherapy with gemcitabine, vinorelbine, and liposomal doxorubicin followed by radiation to a residual supraclavicular node (in India), attaining a complete response. Lacking a sibling or HLA-matched unrelated donor, she was referred to the National Institutes of Health in August 2009 for double umbilical cord blood transplantation (UCBT).

The patient received reduced-intensity conditioning with fludarabine (120 mg/m^2) and cyclophosphamide (4,800 mg/m^2) over 4 days followed by double UCBT on 19 November 2009. Graft-versus-host disease prophylaxis consisted of tacrolimus and sirolimus. Prophylactic antimicrobials included ceftazidime, micafungin, and acyclovir. The patient's pretransplant course was complicated by angioedema, requiring the discontinuation of sirolimus, treatment with high-dose dexamethasone, and admission to the intensive care unit for airway protection. Her immediate posttransplant course was complicated by acute renal failure and pulmonary edema, necessitating continued intubation with mechanical ventilation.

On 24 November 2009, the patient developed distributive/cardiogenic shock. Blood cultures revealed extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*. Fluid resuscitation, vasopressors, and broad-spectrum antibiotics were administered with good response. By 28 November, the patient was off vasopressors but was found to have no purposeful response to stimuli. On 1 December, computed axial tomography (CT) scans of the head and chest were obtained. The head CT was normal. The chest CT revealed a cavitary lung infarction distal to the pulmonary artery catheter. The pulmonary artery catheter tip grew *E. coli*. Bronchoscopy with bronchoalveolar lavage (BAL) was nondiagnostic, but voriconazole was empirically added. Brain magnetic resonance imaging (MRI) on 7 December showed diffuse fluid-attenuated inversion recovery (FLAIR) hyperintensity due to the patient's 100% oxygen requirement but was otherwise unremarkable. A cerebrospinal fluid sample had no white blood cells, and glucose and protein were within normal limits; all microbiological studies were negative. Neutropenia persisted due to delayed engraftment. Graft-versus-host disease prophylaxis at this time consisted of methylprednisolone and tacrolimus. Over the next few days, the patient had only minimal and intermittent central nervous system (CNS) improvement. A chest CT on 14 December showed a new wedge-shaped infiltrate in the left upper lobe of the lung (Fig. 1A1), and liposomal amphotericin B was added. A brain MRI on 17 December showed a focal lesion on the pial surface of the left central sulcus and occipital white matter lesions (Fig. 1A2). A brain biopsy was considered but postponed until the effect of liposomal amphotericin B could be gauged because of the patient's severe thrombocytopenia. A brain MRI obtained on 23 December for continuous mental deterioration revealed considerable progression of the lesions. A chest CT scan on 24 December showed worsening of disease in the left upper lobe (Fig. 1A3). Voriconazole was increased to 8 mg/kg of body weight/12 h and bronchoscopy was repeated. The bronchial wash (BRW) culture grew a filamentous fungus 1 week later.

The patient's neurological status remained unchanged. On 29 December 2009, a brain MRI showed further progression of the lesions (Fig. 1A4) and a brain biopsy was performed. Adequate hemostasis was achieved during surgery, but the patient later developed a large epidural hematoma, distal to the sur-

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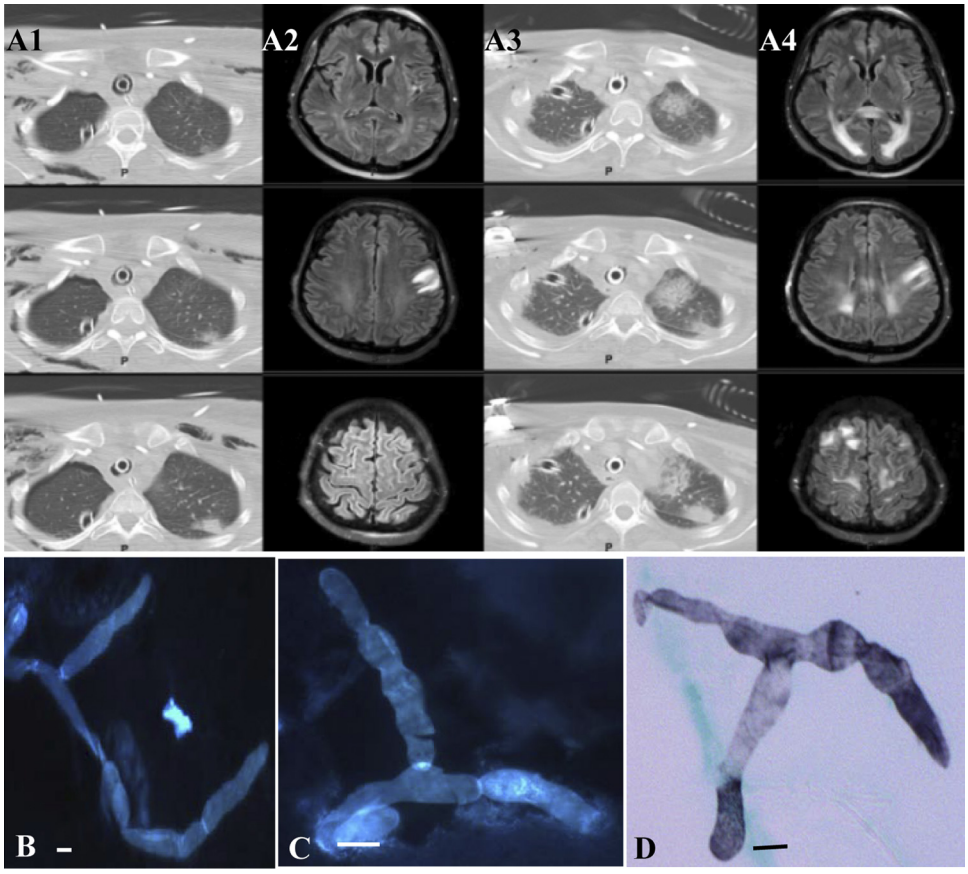

FIG. 1. Computed tomography (CT) of the chest and the magnetic resonance image (MRI) of the brain obtained on four different dates, and *V. volvacea* hyphae in the brain tissue. The CT chest scan on 14 December (A1), MRI brain scan on 17 December (A2), CT chest scan on 24 December (A3), and MRI brain scan on 29 December (A4) are shown. (B to D) The brain biopsy specimen stained with Fungi-Fluor (B and C) and GMS (D) revealed regularly septated, right-angle-branching hyphae (bars, 10 μm).

gical site, with severe midline shift, and died on 3 January 2010. Cultures of the brain biopsy grew abundant colonies of only one type of mold, identical to that isolated from the BRW on 24 December.

Microscopic examination of the brain tissue smear stained with Fungi-Fluor (Polysciences, Inc., Warrington, PA) revealed thin-walled, mostly right-angle-branching septate hyphae of 5 to 11 μm in diameter (Fig. 1B and C). The hyphae also stained positively with Gomori methanamine silver (GMS) stain (Fig. 1D). Fungal isolates from the BRW and brain tissue were each subcultured on Sabouraud agar, potato dextrose agar, malt extract agar (Remel, Lenexa, KS), and alphacel yeast extract agar (6) and incubated at 30°C, 37°C (Fig. 2A), and 40°C. Growth was faster at 37°C and 40°C than at 30°C regardless of the agar medium. Colonies grown for a week on each agar medium were composed of nonsporulating aerial hyphae and substrate hyphae that produced oblong to globose thin walled chlamydospores singly or in chains (Fig. 2B). Upon continuous incubation at 37°C for longer than 2 weeks, the chlamydospores became large (10 to 60 μm in diameter), globose, thick walled, and copper to brown in color (Fig. 2C). As the chlamydospores matured, the colonies turned light orange/gold in color (Fig. 2A). The ultrastructure of the hyphae showed typical dolipore septa with a perforated cap (Fig. 2D, arrow), which is common among the species belonging to *Agaricales* (14). Since the chlamydospore and dolipore septa suggested that the fungus was a basidiomycete but was not definitive for species diagnosis, DNA was isolated from the fungal cultures recovered from the BRW and the brain biopsy specimen for molecular diagnosis. Fungal DNA was also isolated directly from proteinase K-digested brain tissue (25 mg) using the NucliSens easyMAG automated extraction system (bioMerieux, Marcy l'Etoil, France). The internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) (ITS1, 5.8S rRNA, ITS2) was amplified by PCR using the two oligonucleotide fungal rDNA primer pairs ITS1 (5′-TCCGTAGGTGA ACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATAT GC-3′) (16). The sequence of the resulting amplicons was analyzed using the Lasergene software (DNASTAR). Final sequences obtained with each primer set were blasted against the GenBank database (www.ncbi.nlm.nih.gov/BLAST) and realigned relative to best-matched sequences (>90% query coverage and >96% identity) by using MegAlign (DNASTAR) with neighbor-joining analysis. Three sequences, one each from an isolate of the BRW and of the brain biopsy and one directly from the brain tissue, demonstrated 100% homology to each other and 99.8% homology to all three ITS sequences (accession numbers FJ379274, FJ545242, and FJ545239) of

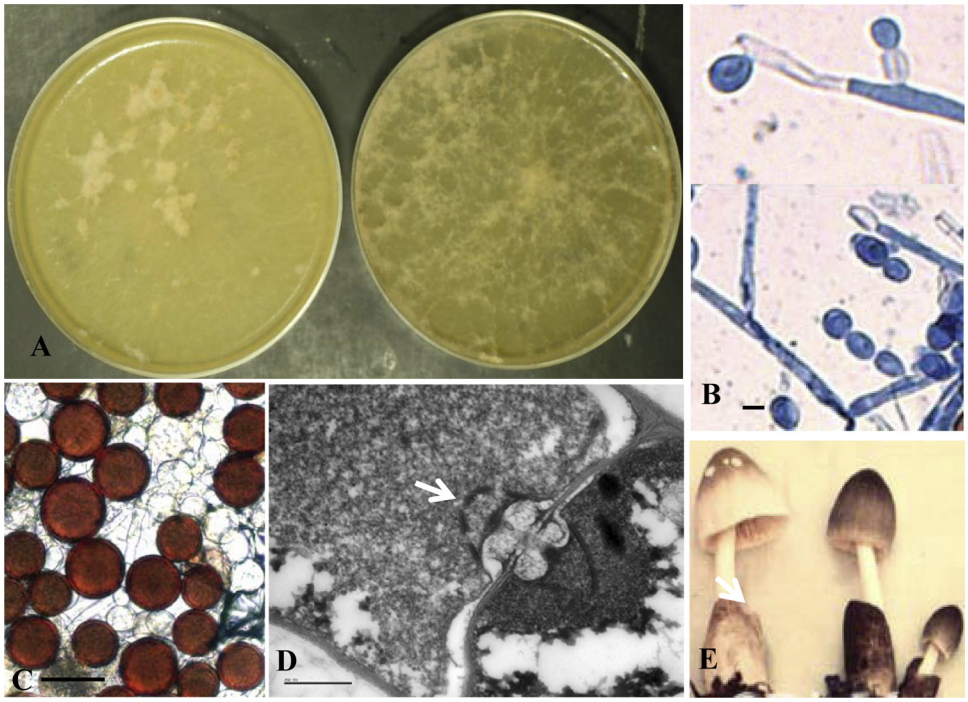

FIG. 2. Morphology of *V. volvacea* grown in laboratory and in nature. (A) Four-week-old colonies grown on alphacel yeast extract agar (left) and malt extract agar (right) at 37°C. (B) Hyphae (no clamp connections) and chlamydospores formed either singly or in chains within a colony grown on malt extract agar for 1 week at 37°C. Cotton blue stained (bar, 10 μm). (C) Mature, globose, thick-walled chlamydospores copper or brown in color in a 4-week-old culture of the brain biopsy isolate grown on alphacel agar (bar, 60 μm). (D) Electron micrograph showing dolipore septa with a perforated cap (white arrow) in hyphae of the brain biopsy isolate grown on malt extract agar for 3 days at 37°C (bar, 500 nm). (E) *V. volvacea* grown in nature (reproduced from BioIdea with permission from Jiankang Jin).

Volvariella volvacea (Fig. 2E) in GenBank. A phylogenetic tree constructed on the basis of ITS sequences of *V. volvacea* strains in the GenBank database and those of the patient isolates is shown in Fig. 3.

Antifungal susceptibility of the patient's isolate (NIH1001) was tested using approved standard M38-A2 of the Clinical and Laboratory Standards Institute (CLSI) broth dilution method (3) at Fungus Testing Laboratory, San Antonio, TX. The test results were reported as MICs (University of Texas Health Science Center [UTHSC] accession numbers 10-91 and 10-92) of amphotericin B (0.5 μg/ml), caspofungin (>8.0 μg/ml), fluconazole (>64 μg/ml), flucytosine (>64 μg/ml), itraconazole (0.5 μg/ml), posaconazole (2 μg/ml), terbinafine (0.125 μg/ml), and voriconazole (2 μg/ml). Other than the MICs for amphotericin B and caspofungin, NIH1001 appeared to be significantly more resistant to antifungals compared to the other filamentous basidiomycetes species (4, 5). No information on the susceptibility of filamentous basidiomycetes against echinocandins is available to compare with that of NIH1001. However, the caspofungin MIC of >8.0 μg/ml for NIH1001 is significantly higher than that reported for ascomycetous fungi, such as *Aspergillus* or *Candida* species (4). Since no other strains of *V. volvacea* are currently available for the comparison of antifungal susceptibility with NIH1001, it is not known whether *V. volvacea* is innately resistant to most antifungals or whether NIH1001 is an unusual mutant. However, it is unlikely to explain how the strain could harbor mutations in several genes conferring resistance to multiple drugs that employ different mechanisms. It should be noted that the clinical relevance of testing antifungal susceptibility in mold infection remains unclear, and breakpoints with proven relevance have yet to be identified or approved by CLSI or any other regulatory agency.

Immune recovery following UCBT is appreciably slower than conventional graft allogeneic transplantation, leading to higher posttransplant infection rates (7, 10, 13). Although im-

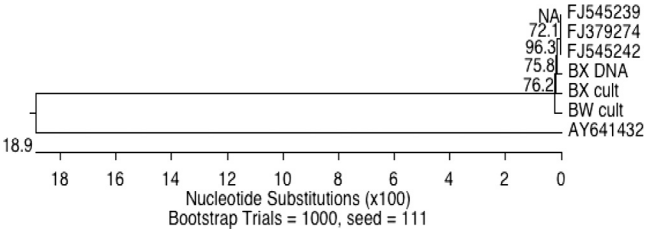

FIG. 3. Phylogenetic tree constructed on the basis of ITS sequences from three environmental strains of *V. volvacea* in GenBank (accession numbers FJ379274, FJ545239, and FJ545242) as well as an isolate from the brain (BX cult), an isolate from the bronchial wash (BW cult), and fungal DNA isolated from the brain tissue (BX DNA) using the neighbor-joining method. Bootstrap analysis with 1,000 replications was performed to establish the tree's robustness. The tree was rooted with a clinical isolate of *Inonotus tropicalis* UTHSC 02–617 (GenBank accession number AY641432) (12).

proved engraftment with the use of new preparative regimens and double cord grafts as well as aggressive supportive care with extended-spectrum antifungals have reduced mortality from treatment-related invasive fungal infection (IFI), IFI continues to be a significant problem during the engraftment period (7). The most common IFIs in immunocompromised patients are pulmonary aspergillosis and yeast-associated fungemia. While human infection by mushrooms is rare, species belonging to *Agaricales*, such as *Schizophyllum commune*, *Coprinopsis cinerea*, and *Inonotus tropicalis*, have been well documented as the cause of systemic infection (6, 12, 15). *Volvariella volvacea* (paddy straw mushroom; Fig. 2E), another member of *Agaricales*, occurs mainly in subtropical and tropical regions of the world, including Southern China, Southeast Asia, and Africa (1, 2). As the species is known to be thermophilic, the patient's isolates grew optimally at 37 to 40°C. *V. volvacea* is a well-known edible mushroom which has long been cultivated in the southern province of China as a delicacy (1). To our knowledge, however, it has not been reported to cause infection in humans or animals. The patient may have inhaled airborne spores of *V. volvacea* in India; India is one of the countries where the species is prevalent, while it is rare in the United States (2). The patient's profound immune impairment associated with delayed engraftment and multiple courses of high-dose systemic steroids may have led to reactivation of the systemic fungal infection. Interestingly, the hyphal morphology of *V. volvacea* in tissue resembled that of mucormycosis with regard to size (hyphal diameter), frequent right-angle branching, and thin walls (6). However, regular septation in the hyphae differentiates it from mucormycotic agents.

Nonsporulating filamentous basidiomycetes such as *V. volvacea* from clinical samples are difficult to identify because the fungus does not fruit on agar medium or produce clamp connections, a hallmark of basidiomycetes (6, 14). Even though some mushroom species, such as *Schizophyllum commune*, may produce fruiting bodies on agar medium, they are often different from those formed in nature (6, 11). Moreover, detailed descriptions of pure cultures of filamentous basidiomycetes grown on conventional mycological medium are lacking (9, 11). The fungus, therefore, may have been underrecognized for its pathogenic potential (8, 9, 11).

Patients undergoing UCBT are highly susceptible to the reactivation of latent fungal infections, especially patients being treated with high-dose systemic corticosteroids. In this case, the patient's infection resembled typical invasive mycoses in immunocompromised patients, with multiple lung and brain lesions. However, it occurred while the patient was receiving micafungin and progressed through liposomal amphotericin B and high-dose voriconazole treatment. When caring for UCBT patients, clinicians must maintain a high index of suspicion for emergent fungal pathogens; diagnostic maneuvers should be pursued so that appropriate treatment may be implemented early.

Nucleotide sequence accession number. The following sequence has been deposited in the GenBank database: *Volvariella volvacea* NIH1001 partial sequence of the 18S rRNA gene; complete sequence of internal transcribed spacer 1, 5.8S rRNA gene, and internal transcribed space; and partial sequence of the 28S rRNA gene (HM367073).

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