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Black mold takes hold and story told

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ABSTRACT

We present a case of an invasive *Curvularia* infection in a patient who developed following bilateral orthotopic lung transplantation despite receiving post-transplant antifungal prophylaxis. This infection presented as mold colonies studding the interior surface of his chest tubes. Despite surgical washout of his bilateral pleural cavities and antifungal treatment with liposomal amphotericin B, micafungin, and isavuconazonium sulfate, the patient died.

1. Introduction

Curvularia species are dematiaceous (melanin-producing) molds that are widespread in the environment. *Curvularia* species are responsible for disease in grasses but can also cause disease in humans [1]. *Curvularia* disease manifestations vary among both immunocompetent and immunocompromised patients. They can present as limited subcutaneous infections [2], keratitis [3], disseminated infections involving multiple organs [4–6], intracerebral abscesses [7,8], invasive fungal rhinosinusitis [9], and peritonitis in patients on peritoneal dialysis [10–12]. Species that have been most commonly recognized to cause human disease include *Curvularia aeria*, *Curvularia geniculata*, and *Curvularia lunata* [1].

Various therapies have been used in patients with infections caused by *Curvularia* species. A study on susceptibility testing on isolates showed amphotericin B, micafungin, and posaconazole were the most active drugs in vitro [13]. However, it should be noted that there are no interpretive criteria for minimal inhibitory concentration (MIC) testing of *Curvularia* species per the Clinical and Laboratory Standards Institute (CLSI) Guidelines.

2. Case

A 67-year-old man from Colombia with end-stage interstitial lung disease was admitted to Duke University Medical Center for bilateral orthotopic lung transplant (BOLT). His BOLT surgery was complicated by hemorrhage requiring multiple units of red blood cells, plasma, platelets, and cryoprecipitate. Post-operatively, his chest was left open, and he was placed on veno-venous extracorporeal membrane oxygenation (ECMO). Per hospital protocol, the patient was placed on inhaled amphotericin B lipid complex 100 mg (mg) daily and intravenous (IV) fluconazole 200mg daily for antifungal prophylaxis with plans to continue through his transplant hospitalization. However, he had worsening liver function, and his fluconazole was switched to micafungin 100mg daily on post-operative day two. On post-operative day two, the patient was also taken back to the operating room (OR) for right middle and lingula lobectomies, and his chest remained open. On post-operative day five, he underwent tracheostomy. On post-operative day seven, the patient's chest was finally closed.

The patient remained critically ill in the intensive care unit with renal failure on continuous renal replacement therapy. He also developed a pneumoperitoneum requiring return to the OR. On post-operative day 53, a black mold was found within and throughout the patient's chest tubes, bilaterally (Fig. 1). Multiple colonies studded the tubes, and their growth appeared to have originated from inside the patient. At this point, he had four chest tubes, all of which had been present since his initial BOLT surgery.

The patient was taken to the OR on post-operative day 54 for washout of the right chest and replacement of chest tubes through a video assisted thoracic surgery (VATS). Mold was encountered throughout his right pleural space and along the pericardium. None was noted along his original clamshell surgical incision from his BOLT. The pleural space was irrigated with 3 L of sterile saline mixed with 10 mL of 4% chlorhexidine gluconate, and chest tubes were replaced. Septate hyphae were seen on KOH preparation from the right pleural tissue. Bacterial and fungal blood cultures were negative as were pleural

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Fig. 1. Multiple colonies of black mold noted from bilateral chest tubes.

cultures for bacteria and mycobacteria and bronchoalveolar lavage fungal cultures. The patient's micafungin was changed to liposomal amphotericin B five mg per kilogram (kg) IV daily. His weekly inhaled amphotericin B lipid complex was continued as well. He was taken again for washout of the left pleural space on post-operative day 56, and no mold was seen.

After the chest tubes were replaced, no further mold was seen in the tubes. The intra-operative tissue cultures from his washout on day 54 grew a dematiaceous filamentous mold identified as *Curvularia* species (Fig. 2). As the patient continued to decompensate, isavuconazonium sulfate 372mg IV every 8 h for six doses then daily thereafter was added

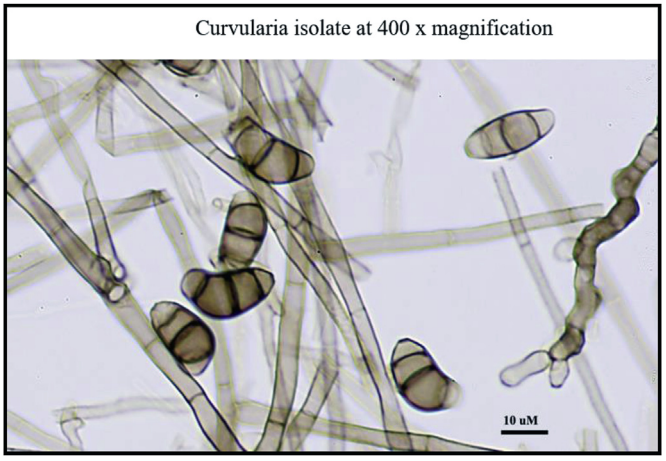

Fig. 2. *Curvularia* isolate at 400 x magnification.

to his therapy. Sixty-three days after transplantation and ten days after detection of invasive mold infection, the patient's family withdrew care due to multiple infectious complications and multiorgan failure.

After the patient died, his fungal isolate was identified as *Curvularia lunata var. aeria*, and susceptibility results returned. Susceptibility testing was performed by the University of Texas Health Science Center at San Antonio. MIC results (micrograms/milliliter) using CLSI Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi were as follows: Amphotericin B 0.06, Micafungin 0.06, Posaconazole 0.125, Voriconazole 8, and Isavuconazole 4.

3. Methods

The fungal isolate was transferred from the Clinical Microbiology Laboratory to the Mycology Research Laboratory for further study, where, based on morphological examination, it was provisionally identified as *Curvularia lunata* [14]. Based on DNA sequence from a portion of the small subunit of the nuclear rDNA repeat, internal transcribed spacer regions 1 and 2, the 5.8S rDNA repeat, and a portion of the large subunit of the nuclear rDNA repeat, the isolate was further identified as *Curvularia lunata var. aeria* (GenBank Accession number MN881067). DNA sequence data was obtained by growing the isolate (DUMC 101.19) on potato dextrose agar under ambient conditions. Next, approximately 0.5 cm-squared of sporulating mycelium was bead milled for 2 min (min) using 0.3 g of glass beads (710–1180 μm) in 700 μL of 200 mM (mM) Tris-HCl (pH 7.5), 250 mM NaCl, 25 mM EDTA, and 1% SDS. DNA was extracted using a phenol:-chloroform:isoamyl alcohol procedure incorporating two 70% ethanol wash steps [15]. Primer pairs ITS1/ITS4 [16], and 5.8SR/LR7 [17], were used under PCR conditions of 3 min at 95 °C followed by 35 cycles of 30 seconds at 95 °C, 3 min at 58 °C, one min at 72 °C, followed by 7 min extension at 72 °C. PCR amplicons were purified using Qiaquick reagents (Qiagen, Valencia, CA). Sequencing, using primers from PCR reactions, was performed by Eton Bioscience (Research Triangle Park, NC). Sequences were edited in Vector NTI ContigExpress (ThermoFisher Scientific, Waltham, MA) and blasted in GenBank (http://www.ncbi.nlm.nih.gov/) using BLASTn to confirm identity.

4. Discussion

In this case report, we describe an unusual presentation of a rare infection. Similar to our case, there is a report of a patient who developed an infection with *Curvularia* after lung transplantation with sputum cultures positive for this organism and a thoracotomy wound with subcutaneous tissues studded with “black mold like material” ([18]). This patient was treated with amphotericin B lipid complex five mg/kg IV daily, Itraconazole 400mg daily, and 5-flucytosine 1250mg daily. The patient underwent several operative debridements of his chest wall but ultimately died of respiratory failure.

Another report describes a sternal wound infection with *Curvularia lunata* in a neonate with congenital heart disease who required urgent corrective surgery on cardiac bypass that included aortic arch repair, implantation of pulmonic valve homograph, and ductus arteriosus ligation [19]. His chest was left open due to cardiac tamponade, and after closure four days later, the patient returned to OR due to recurrent cardiac tamponade. He had a surgical debridement, but *Curvularia* did not grow in culture until after he died, so he did not receive antifungal therapy. In this case, the authors believe that the patient was likely infected through direct inoculation of the wound through environmental contamination. The authors note that the most significant risk factors for this infection were needing two emergency sternotomies and having an open chest for 11 days.

Contaminated operative supplies were implicated in an iatrogenic *Curvularia* outbreak in a series of women with *Curvularia* contaminated saline-filled silicone breast implants [20]. The *Curvularia* in these cases was thought to have been introduced into the saline from a water

damaged ceiling under which the saline bottles were stored. For our patient, environmental sampling of his room did not reveal an apparent source for the *Curvularia*. However, it is highly likely that with the open chest for several days and chest tubes that *Curvularia* seeded the pericardial and pleural spaces and grew despite systemic azole and then echinocandin prophylaxis.

Our patient had many factors that were potentially involved in his *Curvularia* infection. Most notably, he had an open chest for an extended period of time (seven days post-transplant), required two repeat explorations of the chest cavity within the first seven days post-transplant, and had chest tubes that remained in place from time of transplant until 54 (right) and 56 (left) days post-transplant. Other factors that may have contributed to his invasive mold infection included his extended duration on broad-spectrum antibiotics, critical illness with an extended stay in the ICU, and multiple openings into the chest cavity from the outside. Our patient was on both inhaled amphotericin B lipid complex and micafungin during the development of this infection. His *Curvularia* isolate had low MICs for both of these antifungal agents. However, penetration into the pleural space may have been an issue with these. Inhaled amphotericin B lipid complex is preferred over inhaled amphotericin B deoxycholate as both are associated with low rates of pulmonary fungal infections, but inhaled amphotericin B lipid complex has less treatment-related adverse events. However, fungemia, pleural space, and anastomotic infections with both forms have been reported [21]. There are also limited data on the pleural space penetration of IV micafungin, but in a report on concentrations in tissue fluids in seven patients, the micafungin concentration in three patients with pleural effusions was above the MIC_{90} for *Candida albicans, Candida glabrata,* and *Aspergillus fumigatus* when using a dose of 150mg [22].

Our strategy in the management of this was aggressive surgical debridement and systemic antifungal therapy. We chose to add isavuconazonium sulfate (IV) as the patient was critically ill, and we did not want to give IV posaconazole or voriconazole as both IV formulations contain cyclodextrin, and we were concerned about potential accumulation and neurotoxicity with ongoing renal failure. We did not consider administration of antifungals directly into the pleural space; however, this strategy has been used previously in the treatment of fungal empyema in other locations [23].

Given the challenges with treatment, it is critical to prevent these infections. It is important to maintain a clean environment since clearly active antifungal prophylaxis did not prevent introduction of an environmental pathogen into the chest and its development of active disease. In fact, like this case, most of these post-operative black mold chest infections are not easily managed although much of the outcome may relate to multiple surgical interventions both before and after establishment of fungal disease.

Ethical form

We did not obtain the patient's consent for this report before he died. However, we have included a waiver from the Duke IRB attached that allowed us to present this case.

Declaration of competing interest

Dr. Perfect has research grants and is a consultant for: Astellas, Pfizer, Merck, Scynexis, Amplyx, Matinas, Appili and Minnetronix. There are no other conflicts of interest to report.

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