JCM
Journals.ASM.org

Paravertebral Mushroom: Identification of a Novel Species of *Phellinus* as a Human Pathogen in Chronic Granulomatous Disease

Suk See De Ravin,[a] Mark Parta,[b] Deanna A. Sutton,[c] Brian L. Wickes,[d] Elizabeth H. Thompson,[c] Nathan P. Wiederhold,[c] Karen K. Nakasone,[e] Meghna Alimchandani,[f] Amy OConnell,[g] Luigi Notarangelo,[g] Elizabeth Kang,[a] Harry L. Malech,[a] Adrian M. Zelazny[h]

Laboratory of Host Defenses, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA[a]; Clinical Monitoring Research Program, Leidos Biomedical Research, Inc., Frederick, Maryland, USA[b]; Fungus Testing Laboratory, Department of Pathology,[c] and Department of Microbiology and Immunology,[d] University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA; Center for Forest Mycology Research, Northern Research Station, Forest Products Laboratory, USDA Forest Service, Madison, Wisconsin, USA[e]; Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA[f]; Division of Immunology, Boston Children's Hospital, Boston, Massachusetts, USA[g]; Department of Laboratory Medicine Clinical Center, National Institutes of Health, Bethesda, Maryland, USA[h]

We describe a case of paravertebral abscess caused by a *Phellinus* sp. in a boy with chronic granulomatous disease. Sequence-based identification of this mold, a new agent of disease, suggests a close relation to *Phellinus umbrinellus*.

CASE REPORT

A 10-year-old white male with X-linked gp91^{PHOX}-deficient chronic granulomatous disease (CGD) presented for a scheduled clinic visit without any acute complaints. He was diagnosed with CGD (cytochrome b_{245} beta-polypeptide [CYBB] gene) at 17 months of age following presentation with cervical lymphadenopathy. Despite standard prophylactic medications, his course had been complicated by recurrent pneumonias, granulomatous gastric outlet obstruction, and CGD-related inflammatory bowel disease with chronic constipation, perianal fissures and fistula, recurrent abdominal pain, and failure to thrive. His CGD-related prophylaxis medications included cefprozil, itraconazole, and gamma interferon. He also received medications for his gastrointestinal (GI) complications, including cyproheptadine, mesalamine, and topical treatment for perianal disease. Management of his GI disease had periodically included prolonged courses of prednisone (0.1 to 1 mg/kg of body weight/day) prescribed for relapses of perianal disease and gastric outlet obstructive symptoms. His recent history was significant for right hip pain that was not associated with fever, back pain, or focal neurological symptoms. A hip magnetic resonance image (MRI) revealed a small fluid collection in his hip without any specific abnormality.

The discomfort in the hip lasted about 6 weeks and resolved spontaneously without any treatment or analgesics.

On examination, he was afebrile and his height and weight were below the 3rd centile for his age. His physical exam was completely unremarkable. In particular, his cranial nerves and gait were normal, as was the remainder of his peripheral neuromuscular system. His laboratory investigations revealed a white cell count of 138,000/μl, a platelet count of 335,000/μl, a C-reactive protein (CRP) level of 7.4 mg/liter, and an erythrocyte sedimentation rate (ESR) of 21.0 mm/h. Chest computerized tomography (CT), performed as a follow-up for a pneumonia diagnosed and treated a year previously, revealed no new focal pulmonary lesions. However, at the inferior portion of the chest CT (Fig. 1), a right paraspinal mass measuring 3.9 by 2.0 cm at the level of the right kidney with evidence of destruction and erosion of adjacent lower thoracic vertebral bodies was noted. To investigate this lesion further, an MRI of his thoracic/lumbosacral spine was performed and revealed a multilevel right paraspinal mass extending from T11 to L5-S1, with the lesion(s) extending into the neural foramina, though not into the central canal, without evidence of cord compression. Although there appeared to be erosion of the right lateral margins of several lower thoracolumbar vertebral bodies, most notably T11, T12, L1, and L2, no increased uptake was evident on a bone scan. A review of earlier chest CT films revealed evidence of this longstanding lesion, indicating a slow progression of the paravertebral mass. Through this interval, he received empirical combined antimicrobials that included voriconazole, micafungin, levofloxacin, meropenem, and linezolid for treatment for a pulmonary infiltrate of unidentified etiology.

A core biopsy of the lesion was performed under CT guidance, and material was examined for infectious causes as well as malignancies. The histopathology of the paravertebral mass biopsy revealed rare polymorphonuclear leukocytes and necrotizing granulomatous inflammation with abscess formation, and fragmented hyphal forms were seen on a Grocott's methenamine silver (GMS) stain (Fig. 2). Special stains for bacteria (Brown and Hopps stain) and mycobacteria (AFB-Fite) were negative. One colony of mold, later identified as *Phellinus* sp., grew after 12 days of incubation at 30°C.

The paraspinal mass was removed without complication, and voriconazole was continued orally. However, 9 months following surgery, asymptomatic progression of the lesion was again noted by imaging. Following a period of intensified antifungal therapy with addition of posaconazole and caspofungin, a percutaneous aspirate from the progressing paravertebral/psoas abscess again grew *Phellinus* sp. Liposomal amphotericin B was added, and 4 months later, radiographic clearance was observed in an MRI. The patient underwent an allogeneic transplant and has had no signs

Received 6 March 2014 **Returned for modification** 7 April 2014
Accepted 5 May 2014
Published ahead of print 14 May 2014
Editor: D. W. Warnock
Address correspondence to Adrian M. Zelazny, azelazny@mail.nih.gov.

doi:10.1128/JCM.00667-14

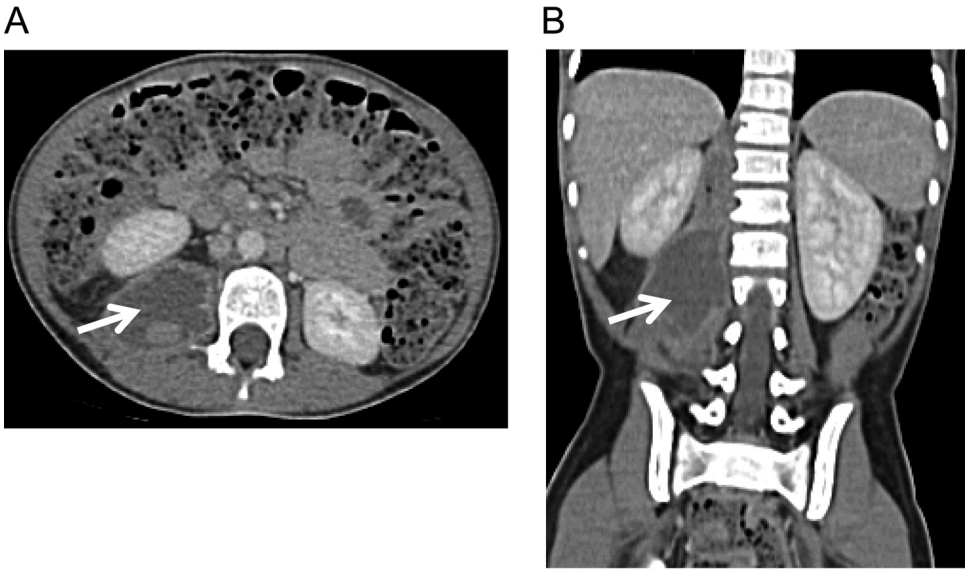

FIG 1 Chest CT revealed a right paraspinal lesion (white arrow) (A), with apparent tethering to thoracolumbar vertebrae evident on coronal section (B).

or symptoms suggestive of recurrence of infection at more than 100 days posttransplantation.

A right psoas mass tissue biopsy specimen collected on 23 August 2012 was processed for bacterial, mycobacterial, and fungal cultures. Direct smears performed in the microbiology laboratory were negative for microorganisms. Bacterial and mycobacterial cultures were negative; however, one small white velvety colony of mold was seen on the inhibitory mold agar (Hardy Diagnostics, Santa Maria, CA) plate after 12 days of incubation at 30°C. The mold grew from one side of the piece of tissue that had been embedded in the agar. Microscopic examination of the colony showed hyaline septate hyphae, but no specific diagnostic features were noted; therefore, amplification and sequencing of the D1/D2 region of the large subunit of the rRNA gene and the internal transcribed spacer (ITS) regions from this strain, designated UTHSCSA DI-13-132, were performed as previously described (1). Sequences were compared with GenBank sequences by means of the nucleotide-nucleotide Basic Local Alignment Search Tool (BLASTn), with significance cutoffs of 90% query coverage and ≥97% identity for potential conspecific sequences. The isolate was similar to *Phellinus cavicola* (GenBank accession no. AY059052.1, 96.5% identity, 587/608 bp match) and *Phellinus umbrinellus* (GenBank accession no. AY059036.1, 96.6% identity, 588/609 bp match) in the D1/D2 region. No significant match (<91% identity) was found in the ITS region. The results of the BLASTn searches for the two loci suggested that the species identity was neither *P. cavicola* nor *P. umbrinellus*. Therefore, the isolate was reported as a basidiomycete most closely related to *Phellinus* sp. and referred to the Fungus Testing Laboratory (FTL) at the University of Texas Health Science Center at San Antonio for further identification and antifungal susceptibility testing.

Over a 9-month period, three fungal isolates collected from

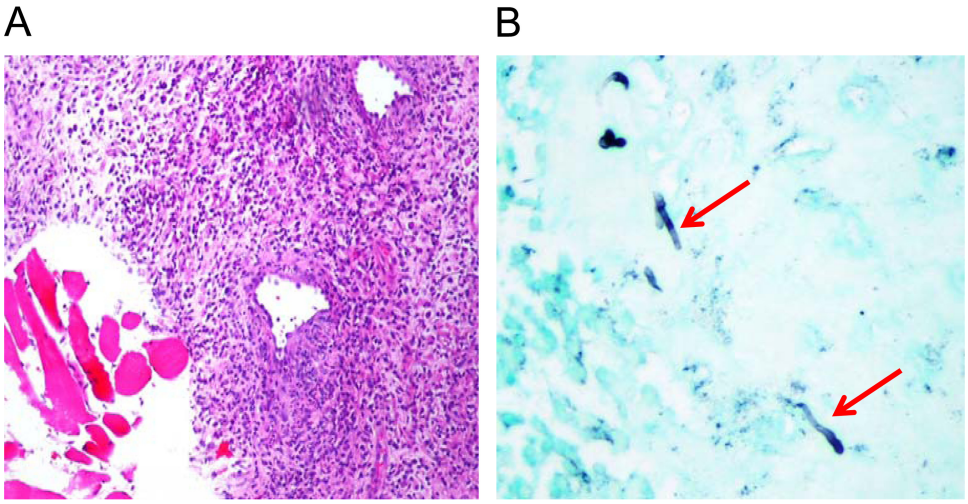

FIG 2 A biopsy specimen of the right psoas muscle shows necrotizing granulomatous inflammation with abscess formation on hematoxylin and eosin stain at a magnification of ×40 (A), which revealed hyphal elements on GMS stain at a magnification of ×400 (B).

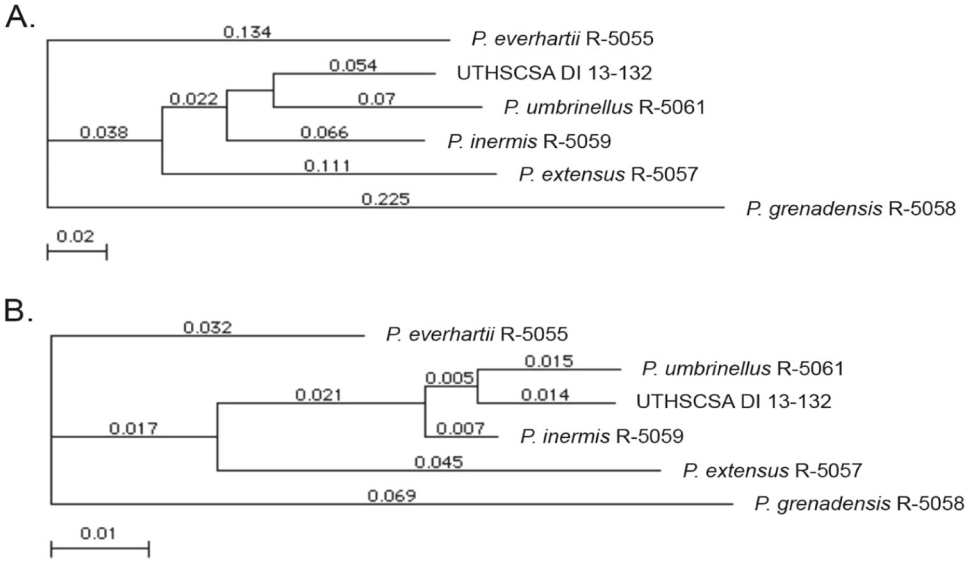

FIG 3 Phylogenetic tree of strain UTHSCSA DI-13-132 and *Phellinus* sp. reference isolates. (A) Analysis of the ITS sequences from UTHSCSA DI-13-132 and the reference isolates showed that UTHSCSA DI-13-132 clustered with *Phellinus umbrinellus* (R-5061). (B) Analysis of the D1/D2 sequences showed that UTHSCSA DI-13-132 again clustered with *Phellinus umbrinellus* (R-5061). Trees were constructed using the neighbor-joining method, and distances were calculated using the Tamura-Nei model (MacVector, Inc., Cary, NC).

this patient were submitted to the FTL for testing. Two isolates that were received by the FTL (UTHSCSA DI-13-131 and UTHSCSA DI-13-132, collected in August and September 2012, respectively) were similar morphologically. Isolates on potato flakes agar (PFA) had a moderate rate of growth, were white to cream (front and reverse), and velvety to floccose. Growth occurred both at 37°C (4+) and at 40°C (1+). Isolates grew on media containing 10% benomyl (2) and failed to grow on 0.04% cycloheximide agar. All media were prepared in house. In addition, the sequences of these first two isolates and a subsequent third isolate from the percutaneous aspirate from the regrowth postsurgery (UTHSCSA DI-13-195, May 2013) were identical. As no diagnostic features were noted in culture, isolates were sequenced and compared with reference strains of *Phellinus* spp. obtained from the Center for Forest Mycology Research, Northern Research Station, USDA Forest Service, Madison, WI (1). These reference strains consisted of *P. everhartii* CFMR-JJW-561 ([UTHSCSA R-5055]), *P. extensus* (MB 101723 [UTHSCSA R-5057]), *P. grenadensis* (CFMR-FP-150348 [UTHSCSA R-5058]), *P. inermis* (CFMR-JLL-15290-sp [UTHSCSA R-5059]), and *P. umbrinellus* (CFMR-FP-105626-Sp [UTHSCSA R-5061]). The ITS and D1/D2 region sequences from the reference strains and strain UTHSCSA DI-13-132 were used to construct a phylogenetic tree (Fig. 3). The results of this comparison suggested that UTHSCSA DI-13-132 was most closely related to *P. umbrinellus*. Without fruit body (basidiocarp/mushroom) formation, which seldom occurs under laboratory conditions, further characterization of these isolates as to whether they are variants of *P. umbrinellus* or a closely related cryptic species is not possible.

Antifungal susceptibility testing was performed against sterile hyphae collected from the three isolates received by the Fungus Testing Laboratory according to the Clinical and Laboratory Standards Institute document M38-A2 guidelines for filamentous fungi (3). For amphotericin B, posaconazole, voriconazole, and terbinafine, the MIC was defined as the lowest concentration of each drug that resulted in 100% inhibition of growth. For caspofungin and micafungin, the minimum effective concentration (MEC) was defined as the lowest concentration that caused abnormal growth characterized by short, stubby, abnormally branched hyphae. Although no antifungal breakpoints have been established against *Phellinus* species, the antifungals amphotericin B, posaconazole, and voriconazole demonstrated *in vitro* activity against each isolate (Table 1). These data corroborate those of earlier *in vitro* studies against a variety of filamentous basidiomycetes, including *Phellinus tropicalis* (4, 5). In contrast, the MECs of micafungin and caspofungin and the MIC of terbinafine were elevated for each isolate. Synergy testing was also performed by the checkerboard technique with the combinations of amphotericin B plus posaconazole and amphotericin B plus voriconazole against the third isolate received by the Fungus Testing Laboratory (UTHSCSA DI-13-195) (6). The fractional inhibitory concentration index (FICI), defined as $FIC_A + FIC_B = (C_A/MIC_A) + (C_B/MIC_B)$, where MIC_A and MIC_B are the MICs of drugs A and B alone, respectively, and C_A and C_B are the concentrations of the drugs in combination, respectively. Although the MIC of amphotericin B decreased from 0.25 μg/ml to ≤0.03 μg/ml when com-

TABLE 1 MICs and MECs for the three isolates sent to the Fungus Testing Laboratory[a]

Agent	MIC or MEC (μg/ml) for:		
	UTHSCSA DI-13-131	UTHSCSA DI-13-132	UTHSCSA DI-13-195
Amphotericin B	0.125	0.125	0.25
Caspofungin	>8	NT	NT
Micafungin	>8	>8	NT
Posaconazole	≤0.03	≤0.03	≤0.03
Voriconazole	0.125	≤0.03	≤0.03
Terbinafine	NT	NT	>2

[a] The MICs (μg/ml) were read as the lowest concentration of drug that resulted in 100% inhibition of growth compared to the growth control, and the MECs were read as the lowest concentration that resulted in abnormal growth. NT, not tested.

bined with posaconazole and voriconazole, these combinations could only be classified as indifferent, as the FICI (1.12) was above the threshold of 0.5 used to classify combinations as synergistic due to the potency of posaconazole and voriconazole (FICI index range for indifferent activity, >0.5 to <4).

CGD is an inherited immune deficiency due to defective NADPH oxidase complex activity that results in an increased susceptibility to infections. In addition to the pathogens that are characteristically seen with CGD, there is an emerging list of microbes that have been identified as pathogens in these patients (7–12). *Phellinus* sp. was isolated and grew out of the psoas muscle *in vitro* from the initial biopsy and again following recrudescence of the lesion after surgical resection. The lesion was discernible on multiple CT images over a year, consistent with the indolent course also described for the three patients reported recently with *Inonotus* (*Phellinus*) *tropicalis* infections (5, 13). Amphotericin was used in all four patients, and surgery was performed in two of them, with resolution of symptoms or radiographic abnormalities in all except for one patient, who remains culture positive despite 7 years of therapy. *In vitro* susceptibilities may provide helpful guidance for the choice of therapeutic agents but are frequently not predictive of a clinical response. An important factor contributing to suboptimal clinical response to antimicrobials is the difficulty of achieving adequate levels of drug, particularly in CGD patients on concomitant medications that may inhibit absorption, in patients with excessive GI losses due to gastrointestinal disease, or in patients who do not comply with prescribed medications. Thus, obtaining drug levels is critical for management of recalcitrant fungal infections. Despite prolonged therapy for months with triple antifungal therapy using azoles and echinocandins prior to surgery, the residual lesion progressed substantially in this patient but responded to prolonged liposomal amphotericin and posaconazole. Although surgery should be considered early for debulking, we believe that in this patient, a combination of oral posaconazole at carefully monitored therapeutic levels and liposomal amphotericin was essential to successful therapy and highlights the importance of a specific microbiological diagnosis.

Previous reports of *I.* (*Phellinus*) *tropicalis* in CGD patients described rapid growth of isolates with yellow-orange color (5, 14), and the first report also described growth on media containing 10% benomyl and 0.04% cycloheximide (14). However, the isolates described here were white and exhibited slow growth over a period of 12 to 14 days. While the isolates in this report grew in the presence of 10% benomyl, no growth was observed on cycloheximide. Failure to grow on media containing cycloheximide is a characteristic of several basidiomycetes (2). Genomic sequencing was essential for the identification of the isolate to the genus level. However, incomplete public databases hindered further identification of this species not previously known as an etiologic agent of human disease. This experience is similar to those of previous reports describing difficulties in the identification of filamentous basidiomycetes as the causative agents of human disease (1, 5, 13, 14).

Nucleotide sequence accession number. The isolate has been deposited into the University of Alberta Microfungus Collection and accessioned as UAMH 11742. Nucleotide data have been deposited with GenBank under accession numbers KF757337 (D1/D2) and KF757338 (ITS).

ACKNOWLEDGMENTS

We thank Christina Henderson and the technologists of the Mycology and Mycobacteriology Laboratory, Microbiology Service, at the National Institutes of Health for their help.

This research was supported by the Intramural Research Program of the NIH Clinical Center.

REFERENCES

1. **Romanelli AM, Sutton DA, Thompson EH, Rinaldi MG, Wickes BL.** 2010. Sequence-based identification of filamentous basidiomycetous fungi from clinical specimens: a cautionary note. J. Clin. Microbiol. **48:** 741–752. http://dx.doi.org/10.1128/JCM.01948-09.
2. **Summerbell RC.** 1993. The benomyl test as a fundamental diagnostic method for medical mycology. J. Clin. Microbiol. **31:**572–577.
3. **CLSI.** 2008. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard, 2nd ed. Document M38-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
4. **Gonzalez GM, Sutton DA, Thompson E, Tijerina R, Rinaldi MG.** 2001. In vitro activities of approved and investigational antifungal agents against 44 clinical isolates of basidiomycetous fungi. Antimicrob. Agents Chemother. **45:**633–635. http://dx.doi.org/10.1128/AAC.45.2.633-635.2001.
5. **Ramesh M, Resnick E, Hui Y, Maglione PJ, Mehta H, Kattan J, Bouvier NM, Labombardi V, Victor TR, Chaturvedi S, Cunningham-Rundles C.** 2014. Phellinus tropicalis abscesses in a patient with chronic granulomatous disease. J. Clin. Immunol. **34:**130–133. http://dx.doi.org/10.1007/s10875-013-9967-1.
6. **Eliopoulos GM, Moellering RC.** 1991. Antimicrobial combinations, p 432–492. *In* Lorian V (ed), Antibiotics in laboratory medicine, 3rd ed. The Williams & Wilkins Co., Baltimore, MD.
7. **Zarember KA, Marshall-Batty KR, Cruz AR, Chu J, Fenster ME, Shoffner AR, Rogge LS, Whitney AR, Czapiga M, Song HH, Shaw PA, Nagashima K, Malech HL, DeLeo FR, Holland SM, Gallin JI, Greenberg DE.** 2012. Innate immunity against Granulibacter bethesdensis, an emerging gram-negative bacterial pathogen. Infect. Immun. **80:**975–981. http://dx.doi.org/10.1128/IAI.05557-11.
8. **De Ravin SS, Challipalli M, Anderson V, Shea YR, Marciano B, Hilligoss D, Marquesen M, Decastro R, Liu YC, Sutton DA, Wickes BL, Kammeyer PL, Sigler L, Sullivan K, Kang EM, Malech HL, Holland SM, Zelazny AM.** 2011. Geosmithia argillacea: an emerging cause of invasive mycosis in human chronic granulomatous disease. Clin. Infect. Dis. **52:** e136–e143. http://dx.doi.org/10.1093/cid/ciq250.
9. **Machouart M, Garcia-Hermoso D, Rivier A, Hassouni N, Catherinot E, Salmon A, Debourgogne A, Coignard H, Lecuit M, Bougnoux ME, Blanche S, Lortholary O.** 2011. Emergence of disseminated infections due to Geosmithia argillacea in patients with chronic granulomatous disease receiving long-term azole antifungal prophylaxis. J. Clin. Microbiol. **49:**1681–1683. http://dx.doi.org/10.1128/JCM.02456-10.
10. **Reichenbach J, Lopatin U, Mahlaoui N, Beovic B, Siler U, Zbinden R, Seger RA, Galmiche L, Brousse N, Kayal S, Gungor T, Blanche S, Holland SM.** 2009. Actinomyces in chronic granulomatous disease: an emerging and unanticipated pathogen. Clin. Infect. Dis. **49:**1703–1710. http://dx.doi.org/10.1086/647945.
11. **Sugui JA, Peterson SW, Clark LP, Nardone G, Folio L, Riedlinger G, Zerbe CS, Shea Y, Henderson CM, Zelazny AM, Holland SM, Kwon-Chung KJ.** 2012. Aspergillus tanneri sp. nov., a new pathogen that causes invasive disease refractory to antifungal therapy. J. Clin. Microbiol. **50:** 3309–3317. http://dx.doi.org/10.1128/JCM.01509-12.
12. **Vinh DC, Shea YR, Sugui JA, Parrilla-Castellar ER, Freeman AF, Campbell JW, Pittaluga S, Jones PA, Zelazny A, Kleiner D, Kwon-Chung KJ, Holland SM.** 2009. Invasive aspergillosis due to Neosartorya udagawae. Clin. Infect. Dis. **49:**102–111. http://dx.doi.org/10.1086/599345.
13. **Davis CM, Noroski LM, Dishop MK, Sutton DA, Braverman RM, Paul ME, Rosenblatt HM.** 2007. Basidiomycetous fungal Inonotus tropicalis sacral osteomyelitis in X-linked chronic granulomatous disease. Pediatr. Infect. Dis. J. **26:**655–656. http://dx.doi.org/10.1097/INF.0b013e3180616cd0.
14. **Sutton DA, Thompson EH, Rinaldi MG, Iwen PC, Nakasone KK, Jung HS, Rosenblatt HM, Paul ME.** 2005. Identification and first report of Inonotus (Phellinus) tropicalis as an etiologic agent in a patient with chronic granulomatous disease. J. Clin. Microbiol. **43:**982–987. http://dx.doi.org/10.1128/JCM.43.2.982-987.2005.