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Invasive Sino-Orbital Mycosis in an Aplastic Anemia Patient Caused by Neosartorya laciniosa

Kathy Malejczyk, Lynne Sigler, Connie Fe C. Gibas, Stephanie W. Smith

Department of Laboratory Medicine and Pathology, Division of Infectious Diseases, University of Alberta, Edmonton, Alberta, Canada; University of Alberta Microfungus Collection and Herbarium, Edmonton, Alberta, Canada; Infection Control, University of Alberta Hospitals, and Division of Infectious Diseases, University of Alberta, Edmonton, Alberta, Canada

We report the first case of Neosartorya laciniosa invasive sinusitis involving the orbit in an immunocompromised male with aplastic anemia. Treatment included surgical debridement with enucleation of the eye and combination voriconazole and micafungin therapy followed by voriconazole alone. The fungus was identified using sequencing of partial bna and calmodulin genes.
accompanied by tissue necrosis were again seen on histopathology of tissue and cortical bone samples, and bacterial cultures were positive for Enterococcus species and CNS. The patient was continued on vancomycin for a total of 6 weeks of therapy. Unfortunately, fungal culture was not requested at surgery, but a repeat serum galactomannan on day 40 remained positive with an index value of 2.075.

Based on our patient’s tenuous clinical picture, we elected to continue him on 300 mg oral voriconazole twice daily and to add 150 mg intravenous micafungin daily based on reported uniform susceptibility to this drug among Neo. and related species classified within Aspergillus section Fumigati (1, 2). A final CT scan done on day 40 documented mucosal thickening within the remainder of paranasal sinuses and no fluid collections. He remained transfusion dependent for his pancytopenia but defervesced and had gradual clinical improvement, resulting in his transfer to a community hospital 13 days later. The patient completed a total of 6 months of antifungal therapy (7 weeks of combination voriconazole and micafungin and 4 months of monotherapy with voriconazole). He was discharged from the hospital 7 months after his initial presentation. He continued on cyclosporine for his aplastic anemia, and his blood counts stabilized. He was scheduled to have reconstructive surgery to his left orbital area. There was no evidence of recurrence of his fungal infection 2 months after stopping voriconazole therapy.

**Mycology.** As per review of the literature, which indicated variable susceptibility to voriconazole depending on species of Neo., the isolate was submitted for further identification to the University of Alberta Microfungus Collection and Herbarium (UAMH), where it was accessioned as UAMH 11627. Subcultures on potato dextrose agar (PDA) (BD) and oatmeal salts agar (OAT; prepared in-house) were incubated at 30°C and 35°C. Growth was faster at 35°C, with the colony on PDA reaching a diameter of 6 cm after 3 days of incubation. The submitting laboratory reported growth at 45°C but no growth at 50°C. Colonies remained yellowish white with no blue-green surface coloration after 14 days. Conidial heads were sparse. The vesicle was about 15 μm wide and bore few phialides on the upper surface. Characteristic yellowish-white, thin-walled ascomata were produced on both media within 7 days but were more profuse on OAT (Fig. 1B). Ascospores were broadly lenticular with two prominent equatorial crests and measured 4.5 to 5 μm long (Fig. 1C). Sequences of the beta-tubulin and calmodulin gene regions were obtained for species identification. DNA was extracted using the EZNA SP fungal DNA kit (United Bioinformatica Inc., Saskatoon, Saskatchewan, Canada). The partial benA and calmodulin genes were sequenced using primers previously described (3, 4). Sequences were edited using Sequencher version 5.0 (Gene Codes Corp., Ann Arbor, MI) and compared with available sequences in the GenBank nucleotide database using a BLAST search. Results were similar for both searches, with the case isolate showing 99 to 100% benA similarity and 98 to 100% calmodulin similarity with several sequences of Neosartorya spinosa and Neosartorya laciniosa, including the ex-type strain KACC 41657. To further assess the genetic relationship between the case isolate and these Neosartorya species, a data set of combined benA and calmodulin sequences was obtained and a parsimony analysis was performed using PAUP* version 4.0b10 (http://paup.csit.fsu.edu/) and with Neosartorya fischeri as the outgroup. The robustness of the trees obtained was evaluated by 1,000 bootstrap replications. In the resultant tree, the case isolate is shown grouping with isolates of N. laciniosa with 100% bootstrap support, and the group is sister to N. spinosa (Fig. 2).

Susceptibility testing performed retrospectively on isolate UAMH 11627 yielded MICs (mg/liter) of 0.5 for amphotericin B, 8 for 5-fluorouracil, >64 for fluconazole, 1 for itraconazole, 0.25 for posaconazole, 1 for voriconazole, and 0.5 for caspofungin.

Invasive aspergillosis (IA) is the most common filamentous fungal infection among immunocompromised patients, with very high mortality rates. Although the majority of infections are attributed to Aspergillus fumigatus, there is increasing recognition of the role of related species in causing invasive disease, especially since the advent of molecular methods allowing for more precise identification of the etiologic agent involved. There are currently nine Aspergillus species and 27 Neosartorya teleomorphs included within the Aspergillus section Fumigati (2) (Mycobank; http://www.mycobank.org/). Neosartorya species are generally recognized by their rapidly growing, poorly sporulating white to pale-green colonies in primary culture, by their thermotolerance (all growing at 37°C and a few up to 50°C), and by formation of ascomata containing ascospores on sporulation media. Currently, Neosartorya hiratsukae, Neosartorya pseudofischeri, and Neosartorya udagawae are confirmed as opportunistic pathogens, with the latter species gaining recent attention (5–13). Although N. fischeri and N. spinosa have been identified in some reports, their roles in causing infection have not been reliably confirmed. Isolates from...
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FIG 2 One of 19 equally parsimonious trees (consistency index, 0.891; retention index, 0.867; homoplasy index, 0.109) inferred from maximum parsimony analysis of combined partial benA and calmodulin gene sequences showing the placement of the case isolate. Bootstrap values above 50% are shown. For each isolate, GenBank accession number and culture collection number are listed. Culture collection acronyms are as follows: CBS, Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; IBT, Culture Collection of Fungi, Technical University of Denmark, Lyngby, Denmark; KACC, Korean Agricultural Culture Collection, Suwon, South Korea; NRRL, USDA Agricultural Research Service Culture Collection, Peoria, IL; UAMH, University Medical Mycology Research Center, Chiba University, Chiba, Japan; NRRL, University of Alberta Microfungus Collection and Herbarium, Edmonton, Canada.

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REFERENCES


4. Some older cases have been reidentified as Neosartorya pseudofischeri, and isolates from more recent cases have not been identified using sequencing of genes appropriate for Neosartorya identification (14–17).

5. Neosartorya infections in humans may include invasive infections of eye, ear, or lung; endocarditis; peritonitis; multifocal brain abscesses; and osteomyelitis (5–16). We report the first case of Neosartorya laciniosa invasive sino-orbital aspergillosis (SOA) in a patient with aplastic anemia. How our patient acquired his infection is unknown. He did not live on a farm or acreage and had not worked for some time due to illness, so no specific exposures could be identified. Neosartorya laciniosa was described in 2006 for 12 isolates from more recent cases have not been identified using sequencing of genes appropriate for Neosartorya identification (14–17).

6. Neosartorya species are uncommonly isolated in our hospital, so there was concern 4 months after this patient’s presentation when a pediatric patient with acute lymphocytic leukemia presented with a necrotic nasal lesion from which a Neosartorya species was again cultured. However, sequencing of the isolate from the second patient determined its identity as N. pseudofischeri.

7. SOA is a relatively uncommon form of aspergillosis, occurring in both immunocompetent and immunosuppressed humans, as well as in canines and felines. Most cases have been described based on clinical and histopathological features, and the etiologic agent has been infrequently identified to the species level (18). A recent study of the etiology of feline SOA in Australia determined that Neosartorya species were the cause of all 17 cases documented (19). Although sequencing of the internal transcribed spacer regions was insufficient to identify the isolates to the species level, the high percentage of similarity, the production of ascospores in some mating studies, and the frequent treatment failure suggest that N. udagawae was the main species involved. N. udagawae differs from N. laciniosa in being heterothallic, in failing to grow above 42°C, and in being refractory to antifungal therapy (10, 12, 13, 19).

8. Our patient was successfully treated with voriconazole and micafungin together with surgical debridement. Antifungal susceptibility testing done retrospectively on the case isolate showed that N. laciniosa is similar to N. hiratsukae in showing low MICs to amphotericin, itraconazole, voriconazole, and caspofungin (1). In contrast, both N. udagawae and N. pseudofischeri show high MICs to voriconazole and itraconazole (1, 10). It is therefore clinically important to not only differentiate Neosartorya isolates from the related Aspergillus species within the section Fumigati but also to identify Neosartorya isolates to the species level.

9. This case confirms that Neosartorya species should be suspected when a white thermotolerant Aspergillus species with sparse conidiation is isolated and that identification to the species level can be obtained by sequencing of partial benA and calmodulin genes. Accurate identification is necessary to elucidate clinical or therapeutic differences between Neosartorya species.

10. Nucleotide sequence accession numbers. Sequences for N. laciniosa UAMH 11627 were deposited in GenBank under accession no. JX845619 for the partial calmodulin gene and JX845620 for the partial benA gene.


