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Acute respiratory distress caused by *Neosartorya udagawae*

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ABSTRACT

We describe the first reported case of acute respiratory distress syndrome (ARDS) attributed to *Neosartorya udagawae* infection. This mold grew rapidly in cultures of multiple respiratory specimens from a previously healthy 43-year-old woman. *Neosartorya* spp. are a recently recognized cause of invasive disease in immunocompromised patients that can be mistaken for their sexual teleomorph, *Aspergillus fumigatus*. Because the cultures were sterile, phenotypic identification was not possible. DNA sequencing of ITS, calmodulin and β-tubulin genes supported identification of *Neosartorya udagawae*. Our case is the first report of ARDS associated with *Neosartorya* sp. infection and defines a new clinical entity.

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1. Introduction

Despite its world-wide omnipresence in soil, *A. fumigatus* had been considered asexual until studies of an Aspergillus-like anamorph isolated from Brazilian soil revealed a heterothallic breeding system, and the teleomorph *Neosartorya fumigata* was described [1]. *Neosartorya* sp. are the complementary mating types of *Aspergillus* and are required for sex to occur. We describe the first reported case of acute respiratory distress syndrome (ARDS) attributed to *Neosartorya udagawae*. The mold grew rapidly in culture of both sputum and bronchoalveolar lavage (BAL) fluid from a previously healthy 43-year-old woman with ARDS, which developed as the consequence of an acute febrile illness that progressed over 5 days. But despite appearance of fluffy white, non-sporulating mold on a variety of culture media, the colonies did not produce conidiophores, and phenotypic identification was not possible. The conidia of *N. udagawae* require longer incubation in the laboratory to germinate, and misidentification of *Neosartorya* sp. as *A. fumigatus* is well described. [2,3] *N. udagawae* has been emerging as a cause of invasive infections in humans, but our case is the first report of ARDS associated with *Neosartorya* infection and describes a new clinical entity.

2. Case

A 43-year-old woman presented to an outlying hospital with complaints of fever and progressive shortness of breath that developed over 5 days. The patient had a past medical history significant for depression managed with sertraline 50 mg per day, and tobacco use (cigarettes). She was brought to her local hospital by the emergency medical service after developing profound weakness, with inability to get out of bed. She was intubated due to respiratory decline and initially required mechanical ventilation with 100% FiO_2 and positive end-inspiratory pressure (PEEP) of 12 mm/H_2O. She was unable to achieve adequate oxygen saturations with mechanical ventilation and required extra corporeal membrane oxygenation (ECMO) at the time of transfer to our facility.

Upon arrival at our facility, the patient was hypothermic (35.9 °C). She was intubated and sedated with a set respiratory rate on mechanical ventilation at 14 breaths/min. Her pulse and

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blood pressure were 79 beats/min and 86/52 mmHg, respectively; the FiO_2 was 99%; her weight was 93 kg (204 lbs). Endotracheal and nasogastric (NG) tube, Foley catheter, and ECMO cannula were in place. Her chest was notable for course breath sounds with diffuse crackles bilaterally. She had palpable distal pulses in all extremities and no signs of peripheral cyanosis. Rashes or signs of trauma were not observed. Neurologic exam was consistent with medically induced paralysis; muscle tone, corneal and pupil responses were all normal. Urine output was adequate.

Complete blood count was notable for leukocytosis of 17.8×10^3 WBC/μL (78% neutrophils), and normocytic anemia (Hgb = 10.7 g/dL). The complete metabolic panel was normal, outside of elevated chloride; hypokalemia (K = 3.1 mg/dL) and depressed protein and albumin (5.4 and 2.6 mg/dL, respectively). An arterial blood gas revealed acidosis and hypoxemia: pH = 7.31; $paCO_2$ = 43 paO_2 = 45; SaO_2 = 80%. Urinalysis was normal, and a urine pregnancy test was negative. Semi-upright portable AP chest X-ray was notable for diffuse alveolar opacities. Endotracheal aspirate and three sets of blood cultures and a urine sample were submitted to the microbiology lab for culture. Serum procalcitonin was 0.17 ng/mL, not consistent with bacterial infection. She was empirically started on Oseltamivir 75 mg q 24 h via the NG, along with intravenous azithromycin and ceftriaxone initiated at the outlying hospital.

During the patient's first 24 h in the ICU, she progressed from hypothermia to a febrile state (39.1 °C), resolving leukocytosis (10.9×10^3 WBC/μL), and an unchanged physical exam. She remained hypoxemic on mechanical ventilation with FiO_2 = 100% and ECMO support. HIV ELISA, mycoplasma serology, and fungal immunodiffusion antibody testing for Aspergillosis, Histoplasmosis, Blastomycosis, and Coccidiomycosis were performed, and all returned negative/non-reactive. A nasopharyngeal swab for multiplex PCR for respiratory viral and intracellular bacterial pathogens (Film Array™, BioFire®, Inc.) was also negative.

On hospital day two, the patient was noted to have a normal body temperature, but remained on paralytics in a medically induced coma. One additional blood culture was obtained, and EBV and CMV serologies were performed; all returned negative. Quantitative immunoglobulins and serum IgE levels were normal. Cultures of an endotracheal tube aspirate (ETA) that were obtained on admission were notable for growth of a fluffy, sterile white mold with yellow center on standard blood agar media.

On hospital day four, endoscopic bronchoscopy was performed. The airways were notable only for mild inflammation. BAL fluid was collected from the left and right lower lobes (LLL & RLL) of the lung and submitted for Gram and Gomori methenamine silver (GMS) staining, which were negative. BAL fluid WBC count was 7076 cells/mm^3. No eosinophils were noted in BAL fluid, and serum eosinophil counts were normal. *Histoplasma* and *Legionella* antigen testing on the BAL fluid was negative, but an *Aspergillus* galactomannan antigen was positive ($\geq$3.75 ng/mL). PCR for influenza (Cepheid® GenXpert™) as well as multiplex PCR for respiratory pathogens (Film Array™, BioFire®, Inc.) were both negative. Again, within 48 h, a similar appearing fluffy, white mold grew in cultures of both LLL and RLL BAL fluid samples on all media (blood agar, Sabouraud agar, and Mycosel™ Agar (Becton Dickinson); a selective medium containing cycloheximide and chloramphenicol to inhibit bacterial growth). Potato dextrose agar (PDA) was inoculated with a sample of mold, but serial lactophenol cotton blue prep exams of the non-sporulating cultures were non-diagnostic, although clearly not consistent with mucormycosis.

Empiric treatment with lipid formulation of intravenous amphotericin B was initiated on hospital day 5 due to failure to improve on broad spectrum empiric antibiotic treatment. Over the next 48 h respiratory status was unchanged and oxygenation parameters and radiographic imaging showed no improvement. On hospital day 7, following two IV doses of liposomal amphotericin B, repeat bronchoscopy was performed. The airways were notable for increased inflammation. BAL fluid was collected from the left lingula and the right middle lobe. The BAL fluid WBC count had decreased to 1182 cells/mm^3. Gram and GMS stains, and histoplasma antigen testing were repeated, and returned negative for yeast, pneumocystis or fungal elements. *Aspergillus* galactomannan antigen ELISA remained positive (1.99 ng/mL). Methylprednisolone 125 mg IV every 8 h was added to the empiric IV amphotericin B for treatment of possible allergic bronchopulmonary aspergillosis (APBA). Mold was not cultured from either BAL fluid sample on PDA or Sabouraud agar after 45 days of incubation at 30 °C, or blood agar at 37 °C.

Our patient slowly improved and intravenous (IV) methylprednisolone was tapered from 125 mg every 8 h to 40 mg twice a day over a 7 day period. On hospital day 15, methylprednisolone was discontinued and empiric antifungal treatment was changed from IV amphotericin B to voriconazole 40 mg IV every 12 h. On hospital day 18, ECMO treatment was terminated, and our patient was weaned successfully from mechanical ventilation and extubated. She was transitioned to nasal cannula oxygenation and participated in physical therapy. She was continued on voriconazole, but developed transaminitis prompting cessation of the drug due to hepatotoxicity. Further antifungal treatment or steroid treatments were not administered. On hospital day 27, our microbiology laboratory and a state reference laboratory independently identified our mold as *Aspergillus fumigatus* employing phenotypic criteria (Fig. 1).

Partial calmodulin, β-tubulin, and ITS gene sequences were obtained via PCR amplification of genomic DNA extracted from our clinical isolate. Based on DNA sequencing, the mold was identified as the morphologically identical but phylogenetically distinct species *Neosartorya udagawae* (Tables 1–3). As a result of profound deconditioning, our patient was transferred on day 28 to an inpatient unit to complete intensive physical therapy. Subsequently, she returned to work and had no evidence of recurrent infection on follow-up examination.



Fig. 1. NRRL 62810, the mold grown from right lower lobe BAL fluid sample. (A) Colony grown 5 days on malt extract agar, (B) vesicle and phialides resembling *A. fumigatus*, bar = 10 μm.

Table 1
GenBank matches (sorted by identity) for ITS sequence (GenBank accession number KJ572798 – 373 bp).

Gene	GenBank reference organism	GenBank accession number	Length	Identities	Gaps	Max score/total score
ITS	*Neosartorya fisheri* isolate UFMGCB 6414	KJ471558.1	530	372/373	0/373	684/684
ITS	*Aspergillus wyomingensis* strain CCF 4417	HG324081.1	837	372/373	0/373	684/684
ITS	*Neosartorya udagawae* strain ATCC MYA-4693	HQ263363.1	571	372/373	0/373	684/684
ITS	*Neosartorya udagawae* strain ATCC MYA-4691	HQ263362.1	537	372/373	0/373	684/684
ITS	*Neosartorya udagawae* strain ATCC MYA-4690	HQ263361.1	531	372/373	0/373	684/684

Table 2
GenBank matches (sorted by identity) for β-tubulin sequence (GenBank Accession number KJ572796 – 798 bp).

Gene	GenBank reference organism	GenBank accession number	Length	Identities	Gaps	Max score/total score
β-Tubulin	*Neosartorya udagawae* CBM FA-0702	AB248302.2	510	508/510	0/510	942/942
β-Tubulin	*Neosartorya udagawae* isolate KACC F3759	DQ534103.1	509	508/508	0/508	939/939
β-Tubulin	*Neosartorya udagawae* isolate FH103	DQ058395.1	457	457/457	0/457	845/845
β-Tubulin	*Neosartorya udagawae* isolate FH237	DQ058394.1	454	454/454	0/454	839/839
β-Tubulin	*Neosartorya udagawae* strain CBS 154.89	DQ534080.1	507	506/507	1/507	929/929

Table 3
GenBank matches (sorted by identity) for Calmodulin sequence (GenBank Accession number KJ572797 – 743 bp).

Gene	GenBank reference organism	GenBank accession number	Length	Identities	Gaps	Max score/total score
Calmodulin	*Neosartorya udagawae* calmodulin gene, partial cds	KF703499.1	537	537/537	0/537	992/992
Calmodulin	*Aspergillus* sp. IFM 54302	AB259969.1	508	508/508	0/508	939/939
Calmodulin	*Aspergillus* sp. CBM FD-0143	AB259970.1	507	507/508	1/508	939/939
Calmodulin	*Aspergillus* sp. IFM 53867	AB259972.1	507	507/508	1/508	931/931
Calmodulin	*Neosartorya udagawae* strain CCF 4233	HG426054.1	662	661/663	1/663	1212/1212
Calmodulin	*Neosartorya udagawae* isolate KACC 41683	DQ534176.1	547	543/545	2/545	994/994

Table 4
Phenotypic and molecular identification of mold in five specimens.

Specimen	DOT[a]	Phenotypic identification	PCR/ESI-MS	DNA sequencing
ETA[b]	0	*Aspergillus fumigatus*	–	
Right LL[c] BAL fluid	0	*Aspergillus fumigatus*	–	*Neosartorya udagawae*
Left LL[c] BAL fluid	0	*Aspergillus fumigatus*	–	–
Right ML BAL fluid	2	–	*Neosartorya* sp.	–
Left lingula BAL fluid	2	–	*Neosartorya* sp.	–

[a] Days of antifungal treatment.
[b] Endotracheal tube aspirate.
[c] Lower lobe.

3. Discussion

Neosartorya spp. are the complementary mating types of *Aspergillus* and are required for sex to occur. They have a similar microscopically appearance to *A. fumigatus*. Although *Neosartorya* infections are uncommon, cases of *Neosartorya* infection misidentified as *A. fumigatus* are well represented in the literature, suggesting the incidence of *Neosartorya* infections may be higher than suspected [3]. In a retrospective evaluation of specimens banked from patients diagnosed with invasive pulmonary aspergillosis at NIH between 2000 and 2008, Vinh et al. [2] conducted multilocus DNA sequencing to re-examine 36 cases of infection attributed to *A. fumigatus* based on phenotypic identification. Of these, four cases were found to be caused by *N. udagawae*; three were seen in patients with chronic granulomatous disease, and one was in a patient with myelodysplastic syndrome. [2] The median duration of illness was approximately seven times longer than that typically observed for illness caused by *A. fumigatus*. The MICs of various antifungals for the *N. udagawae* isolates were higher than those for *A. fumigatus*, and the disease caused by *N. udagawae* was refractory to standard therapy [2].

To make a precise identification of the mold cultured from our patient, DNA was extracted and partial ITS, β-tubulin and calmodulin genes were amplified with "touchdown" PCR with the annealing temperature running from 55 to 45 °C, and sequences were generated. An NCBI Blast query of the 798 bp partial β-tubulin gene sequence against the NCBI GenBank nr/nt nucleotide database returned six *N. udagawae* isolates with 100% similarity, and returned 28 *N. udagawae* and seven *Aspergillus* sp. sequences at the 99% similarity level (Table 1). A query of the 373 bp partial ITS gene sequence returned seven *N. udagawae* with 100% similarity (Table 2). A query of the 743 bp partial calmodulin gene sequence returned five *Aspergillus* sp. with 100% similarity, and 10 *N. udagawae* and one *Aspergillus* sp. hits at 99% similarity (Table 3).

Sequences from type strains of species near *N. udagawae*, high similarity sequences found in the BLAST search, and the sequences from our clinical isolate were aligned and analyzed with MEGA 5.2. The resulting phylogenetic trees (Fig. 2) show large diversity among *N. udagawae* isolates and reveal that our isolate is in the *N. udagawae* clade. The *Aspergillus* sp. sequences with identical or high similarity sequences to *N. udagawae* represent asexual colonies of this heterothallic species. Because the species is

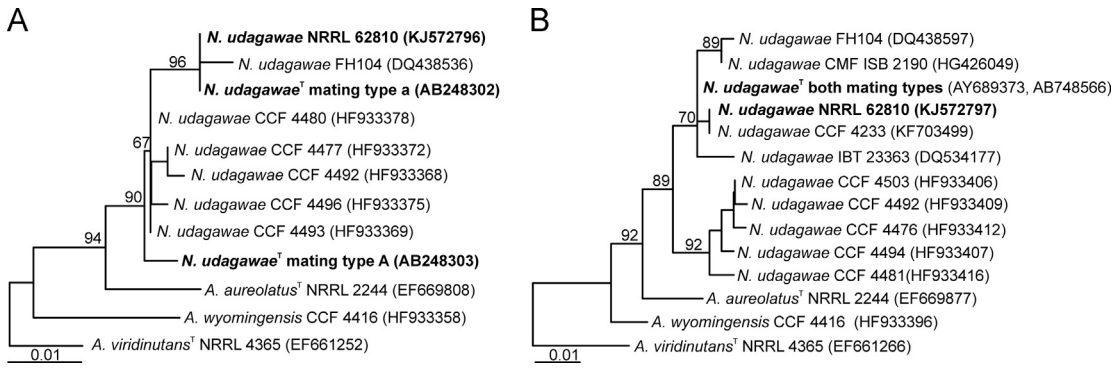


Fig. 2. Phylogenetic trees of (A) beta-tubulin and (B) calmodulin sequences calculated with maximum likelihood in MEGA5.2. Our isolate NRRL 62810 from the patient clearly fits into the *Neosartorya udagawae* clade. Strong bootstrap values on subclades of *N. udagawae* isolates suggest that a more rigorous examination of the isolates would show that *N. udagawae* is a species complex with diagnosable subcomponents. Strain numbers and GenBank accession numbers follow the species names.

heterothallic, it will most commonly be recovered as the *Aspergillus* phase of the species. The morphology of the *Aspergillus* phase is very similar to *A. fumigatus* and the definitive identification is through DNA sequencing and phylogenetic analysis (Tables 1–3).

Histopathology and culture, ideally in combination, are the current basis for diagnosis and identification of fungal infections. Our patient's isolate only produced diagnostic conidiophores after 12 days of incubation, and then it was misidentified as *Aspergillus fumigatus* by two microbiology laboratories. Although it took a week to grow the mold in culture and extract DNA to definitively identify the isolate as *N. udagawae* using ITS, β-tubulin and calmodulin sequence data, the identification could have been done in 2–3 days if genomic DNA had been extracted directly from the original slant culture. Prompt identification of invasive molds directly from clinical specimens is not feasible by conventional microbiologic techniques, but molecular methods for rapid detection of molds directly from BAL fluid offer the potential opportunity to direct antifungal treatment and guide evaluation of underlying host susceptibility. PCR/ESI-MS is capable of rapid identification of fungal pathogens directly from clinical specimens [4,5]. Institutional IRB approval was obtained for submission of samples of our patient's BAL fluid for testing by PCR/ESI-MS, as described by Shin et al. [4]. BAL fluid from the first bronchoscopy was fixed for cytology testing and unavailable for PCR/ESI-MS testing. PCR/ESI-MS was performed on BAL fluid from the second bronchoscopy, which was performed on hospital day 7, 48 h after initiation of amphotericin B treatment. PCR/ESI-MS detected a *Neosartorya* sp. from both RML and left lingular BAL fluid specimens. Fungal cultures were completely negative (Table 4).

Although airborne conidia of *Neosartorya* sp. are conceivably capable of causing an APBA-like syndrome that is seen with *A. fumigatus* in asthmatic patients, this has not been described. Our patient did not have a history of asthma, nor did her laboratory testing exhibit peripheral eosinophilia or elevated IgE titers suggestive of APBA. Her response to steroid treatment is consistent with what would be expected in patient with the fibroproliferative stage of ARDS.

ARDS requiring ECMO treatment associated with *Neosartorya* sp. infection has not been previously described. Due to uncertainty about the species of mold responsible for the pulmonary infection in our patient, she was unnecessarily exposed to toxic empiric antifungal treatment with amphotericin B. More timely identification of the pathogen would have resulted in earlier initiation of pathogen specific directed therapy. While colonization is always a concern with clinical cultures from intubated patients, identification of *N. udagawae* by gene sequencing of mold grown from multiple lobes in both lungs and by PCR/ESI-MS directly from BAL fluid samples taken *after* initiation of antifungal treatment strongly supports the biologic plausibility of *N. udagawae* as the etiology of ARDS in our patient. Finally, given that the MICs of most antifungals for *N. udagawae* isolates are higher than for *A. fumigatus*, and that disease caused by *N. udagawae* can be refractory to standard therapy, rapid recognition of *N. udagawae* as the cause of disease can be critical [2].

Presently, it is unclear how often *Neosartorya udagawae* and other species within *Aspergillus* section *Fumigati* cause invasive pulmonary or sinus infections, or allergic bronchopulmonary or sinus manifestations. As molecular modalities such as β-tubulin and calmodulin sequencing, and PCR/ESI-MS become integrated into diagnostic clinical microbiology laboratories, detection of putatively less common invasive molds such as *Neosartorya* sp. causing pulmonary disease may increase, thereby providing a more detailed picture of the spectrum of mycoses that infect humans and other animals.

Conflict of interest

R. Sampath and K.S. Lowery are salaried employees of Ibis Biosciences, a division of Abbott.

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