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## Diagnostic Microbiology and Infectious Disease

journal homepage: [www.elsevier.com/locate/diagmicrobio](http://www.elsevier.com/locate/diagmicrobio)A case of allergic fungal rhinosinusitis due to *Ceratocystis adiposa*☆Kshitij Agarwal<sup>a</sup>, Shallu Kathuria<sup>b</sup>, Gandhi Sundar<sup>b</sup>, Prithviraj Singh<sup>c</sup>,  
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## ABSTRACT

*Ceratocystis adiposa* known as phytopathogen of conifers has not been recognized so far as a human pathogen. Herein, we report for the first time a case of allergic fungal rhinosinusitis due to *C. adiposa*. The fungus was identified by sequencing internal transcribed spacer of rDNA and D1/D2 of larger subunit region.

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## 1. Introduction

Allergic fungal diatheses of the upper respiratory tract presents as allergic fungal rhinosinusitis (AFRS), while that of the lungs manifests as allergic asthma and allergic bronchopulmonary mycosis (ABPM). Interestingly, in cases of ABPM, the aspergilli are universally considered *sine qua non*; the same cannot be extrapolated to AFRS despite these two being the outcome of the same disease process. Although in the western hemisphere, dematiaceous moulds are the most common agents responsible for AFRS, whereas *Aspergillus flavus* is usually the etiologic agent in the Indian subcontinent and the middle-east (Chakrabarti et al., 2009). The fungi usually associated with AFRS globally include species of *Curvularia* spp., *Bipolaris* spp., *Exserohilum* spp., *Alternaria alternata*, *Schizophyllum commune*, *Pseudallescheria boydii*, etc (Chowdhary et al., 2013b; deShazo and Swain, 1995). Rarely, *Rhizomucor* (Goldstein et al., 1992), *Epicothium nigricans* (Noble et al., 1997), *Trichoderma longibrachiatum* (Tang et al., 2003), *Neosartorya hiratsukae* (Shivaprakash et al., 2009), etc, have been associated with AFRS. Herein, we present the case of AFRS due to *Ceratocystis adiposa*, a plant pathogen, which to the best of our knowledge, has not been reported as an agent of human disease so far.

## 2. Case report

Our patient was a 60-year-old man, sugarcane farmer from Uttar Pradesh, India. He presented in December 2012 with the chief complaints of recurrent sneezing, bilateral anterior rhinorrhoea, and persistent post-nasal drip for the past 30 years, which had worsened over the last 5–6 years. His complaints were worse in the winters. He denied any history of breathlessness, wheezing, eczematous rashes, or any other symptoms suggestive of an atopic process. He had been receiving symptomatic treatment comprising of nasal decongestants, antihistamines, and inhaled steam through primary care physicians on and off but had never been comprehensively investigated in the past. Anterior rhinoscopy revealed bilaterally pale nasal mucosa with mucoid discharge. A computed tomography (CT) of the sinuses showed accumulation of hyperdense mucus in the right maxillary sinuses along with mucosal thickening of the ethmoid sinuses bilaterally (Fig. 1). Given the association of 'hyperdense' mucus with allergic fungal sinusitis, he was investigated for the presence of the same. His chest radiograph was normal. The haemogram revealed a total leucocyte count of 8214 cells/ $\mu$ L of which 9% cells were eosinophils. The absolute eosinophil count was noted to be 700 cells/ $\mu$ L against a normal value of <350 cells/ $\mu$ L used in our Institute. The total serum immunoglobulin E (IgE) level was 2887 IU/mL against a pooled serum value of <200 IU/mL. Precipitating antibodies against *Aspergillus fumigatus*, *A. flavus*, *Aspergillus tamari*, *Aspergillus niger*, *Aspergillus terreus*, *Alternaria alternata*, *Bipolaris hawaiiensis*, *Curvularia lunata*, *Scedosporium apiospermum*, and *Schizophyllum commune* were not demonstrated on the Ouchterlony immunodiffusion test

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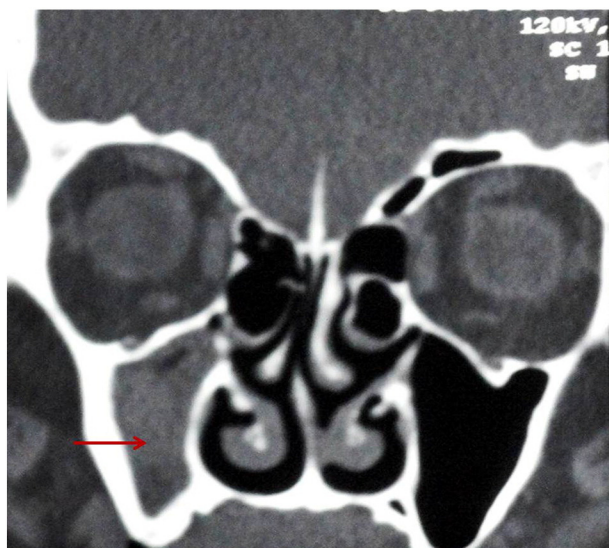


**Fig. 1.** A noncontrast CT of the paranasal sinuses demonstrating opacification of the right maxillary sinus (arrow) with hyperdense material.

using the culture-filtrate antigen of the above-mentioned fungi (Chowdhary et al., 2012). Similarly, skin prick tests and specific IgE to these fungi were negative. His endoscopic evaluation revealed thick mucin with polypoidal mucosa in the meati bilaterally. An antral lavage was done, and antral wash samples were sent for histopathological and mycological examination. Also, nasal washings were performed on 3 separate occasions and were processed for mycological investigations. A functional endoscopic sinus surgery was advised but was declined by the patient. Histopathological examination of the tissue confirmed the presence of allergic mucin (Fig. 2). The diagnosis of allergic fungal sinusitis was made, and the patient was managed using self-administered saline irrigation and intranasal mometasone 100 µg twice a day for 3 months. The latter was then reduced to 50 µg twice a day in addition to the saline irrigations. Upon last contact with the patient 3 months back, he reported symptomatic relief and is presently being managed with intranasal mometasone.

Direct potassium hydroxide (KOH) wet mount of the patient's nasal and antral wash specimens revealed hyaline septate hyphae (Fig. 3A). Also, Periodic acid Schiff (PAS) stain of antral wash specimen was positive for septate hyphae (Fig. 3B). Multiple cottony black mould colonies were isolated on Sabouraud's glucose agar (SGA) after

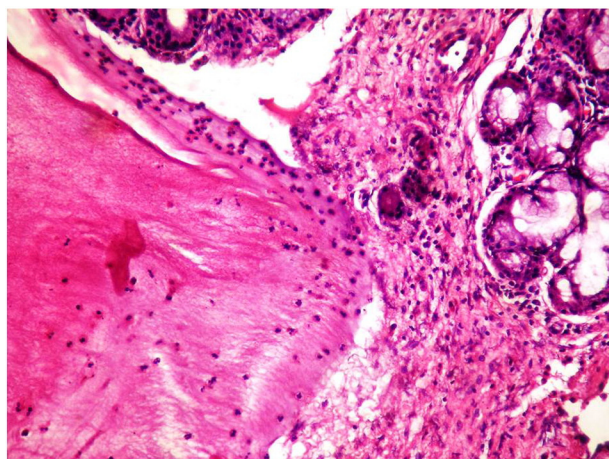


**Fig. 2.** Section showing nasal mucin glands, inflammatory infiltrate comprised of lymphocytes, plasma cells, and eosinophils with PAS positive mucin (left), ×400.

4 days of incubation at 28 °C (Fig. 3C). Slide cultures on SGA showed brownish septate hyphae with dark brown spherical to oblong conidia measuring 5–8 × 7.5–12 µm in chains with basipetal succession at the tip of phialids (Fig. 3D). The isolate was assigned the accession number VPCI2818/12. The isolate was identified by sequencing of the internal transcribed spacer (ITS) region of rDNA and D1/D2 domain of the larger subunit (LSU) region (Chowdhary et al., 2013a). GenBank BLAST searches of ITS and LSU sequences of our isolate exhibited 99% identity with *C. adiposa* sequences available in GenBank with accession numbers EU918711.1 and AY283562.1, respectively. The ITS and LSU sequences of our isolate were submitted to GenBank (Accession numbers KF060719 and KF060720).

### 3. Discussion

*C. adiposa* is an ascomycete mould, responsible for blue stain of conifers, Dutch elm disease, oak wilt, etc (Guzmán-López et al., 2007). This species was first described from sugarcane culms from India as *Sphaeronema adiposum* in 1906 (Butler, 1906). Both Hunt (1956) and Griffin (1968) placed *C. adiposa* under the group in which the ascospores are unsheathed. However, Sartoris (1927) and Bhat (1972) later reported sheathed ascospores of *C. adiposa*. Our patient had been suffering from persistent sinus disease not responsive to routine medication, which is the usual presentation of AFRS. The evaluation for AFRS was prompted by the visualization of 'hyperdense' mucus impaction in the maxillary sinus, which is characteristic of allergic mucin (Panjabi and Shah, 2011). Peripheral blood eosinophilia and raised total IgE supported an allergic diathesis. Our patient fulfilled all the criteria needed to diagnose AFRS proposed by deShazo and Swain (1995), namely, radiological evidence of sinusitis; presence of 'allergic mucin' in the sinus; demonstration and culture of fungal elements in the surgically obtained material and absence of invasive fungal disease and immunosuppression. However, the patient could not be tested for the presence of immediate or delayed skin reactions or serological tests specific to the mould as no antigens were available. The repeated isolation of *C. adiposa* from the nasal secretions and sinus material indicated towards the same as the aetiological agent. In the Indian context, *A. flavus* is the most commonly implicated agent of AFRS (Chakrabarti et al., 2009) probably due to the large size (3–6 µm) of the conidia/spores, which are retained at the upper airways (Pasqualotto, 2009). The large sized spores of *C. adiposa* suggest a similar scenario. Given the association of this mould with sugarcane, it seems likely that the patient repeatedly inhaled spores of *C. adiposa* during his work as a sugarcane farmer. Although one may argue that this association should have led to more widespread reporting of this mould as an agent of AFRS, it may be pointed that the identification of fungi requires the employment of molecular techniques, which are not available in the routine microbiological diagnostic laboratories in India. Further credence to this aetiological association was provided by the negative skin tests and serology against other fungi implicated in AFRS. Moreover, our patient did not give any complaints indicative of a pulmonary affliction, which was supported by a normal chest radiograph thereby negating the possibility of a sino-bronchial allergic mycosis (Venarske and deShazo, 2002). Thus, the ascomycete mould *C. adiposa* is reported as human pathogen for the first time, which extends the spectrum of aetiological agents responsible for AFRS.

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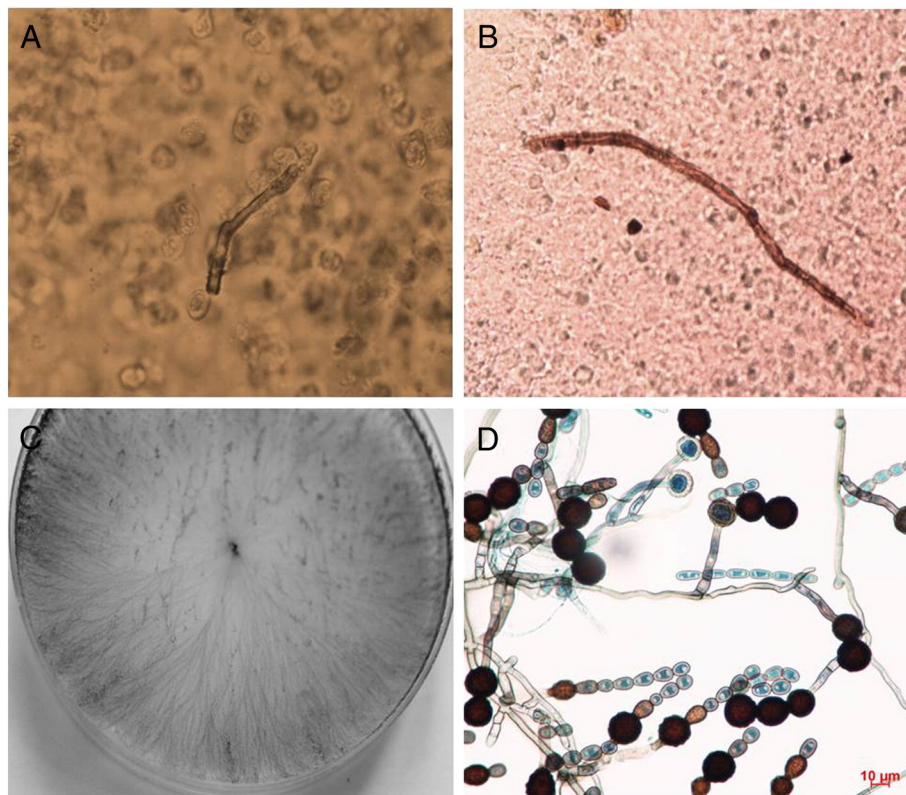


**Fig. 3.** (A) KOH wet mount of patient's antral wash showing hyaline septate hyphae. (B) PAS stain of smear from patient's nasal secretion showing presence of septate hyphae. (C) Patient's isolate VPCI 2818/12 showing cottony black mold colonies on SGA plate after 4 days of incubation at 28 °C. (D) Slide cultures on SGA showing brownish septate hyphae with dark brown spherical to oblong conidia (5–8 × 7.5–12 μm) in chains at the tip of phialids, ×400.

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