

Unusual causes of fungal rhinosinusitis: A study from a tertiary care centre in South India

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Abstract

Purpose: The frequency of mycotic infections of the nose and paranasal sinuses has been increasing over the past three decades. Apart from the common causes of fungal rhinosinusitis such as *Aspergillus* species and *Penicillium* species, there have been reports of rare and unusual fungi isolated from India and other countries. **Objective:** The objective of this study is to find out the prevalence of fungal infections of the nose and paranasal sinuses caused by unusual fungal isolates at a tertiary care teaching hospital in South India. **Materials and Methods:** Duration of the study period was from April 2009 to March 2010. Specimens were collected from the nose and paranasal sinuses of all clinically and radiologically diagnosed cases of rhinosinusitis. All the clinical specimens were processed by standard methods for fungal culture. This included initial screening by 10% potassium hydroxide, inoculation of the specimen onto Sabouraud dextrose agar and incubation at 25°C and 37°C, followed by slide culture and other special techniques wherever necessary. Histopathological examination was also performed for the specimens. **Results:** A total of 60 specimens were received for fungal culture from cases of rhinosinusitis during the period, out of which 45 showed no growth. There were nine cases of *Aspergillus flavus*, 1 each of *Aspergillus fumigatus* and *Penicillium* species. The rest four specimens grew rare fungal isolates, i.e. *Acremonium* sp., *Scedosporium apiospermum*, *Cladosporium cladosporioides* and *Lasiodiplodia theobromae*. Histopathological findings were also positive for these four cases. **Conclusion:** Apart from the common causes, unusual fungal pathogens were isolated from cases of rhinosinusitis during the study period, which is in accordance with similar reports from other parts of India and some other countries.

Key words: *Acremonium*, *Cladosporium*, fungal rhinosinusitis, itraconazole, *Scedosporium*

Introduction

Fungal rhinosinusitis (FRS) is broadly defined as the spectrum of pathologic conditions associated with sinonasal inflammation that is related to the presence of fungi. The frequency of mycotic infections of the nose and paranasal sinuses in India has been increasing over the past three decades.^[1,2] This may be due to a number of factors like increased awareness about fungal infections, improved diagnostic facilities in mycology and factors which can

lead to immunosuppression like increased usage of broad spectrum antibiotics and corticosteroids, increase in the prevalence of chronic diseases such as diabetes, malignancy and human immunodeficiency virus infection.

FRS is broadly classified into invasive and non-invasive types depending upon invasion of the mucosal layer.^[3] Invasive FRS includes: acute invasive FRS, chronic invasive FRS and granulomatous invasive FRS. The noninvasive diseases include: localised fungal colonisation of the nasal and paranasal sinus mucosa, fungal ball and fungus-related eosinophilic FRS that includes allergic fungal rhinosinusitis (AFRS).^[3,4] Apart from the common causes of FRS such as *Aspergillus* spp, *Penicillium* spp, *Fusarium* spp etc., there have been reports of rare fungal isolates from India and other countries.^[5-9] This study was conducted with the objective to find out the prevalence of fungal infections of the nose and paranasal sinuses caused by rare or unusual fungal isolates at a tertiary care teaching hospital in South India.

Materials and Methods

Duration of this study period was from April 2009 to March 2010. All patients attending the out-patient department (OPD) of the E.N.T. Department of Sri Ramachandra Medical College and Research Institute (SRMC and RI), Chennai and clinically diagnosed as cases of rhinosinusitis were included in the study. All these

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patients with a clinical diagnosis of rhinosinusitis underwent a thorough evaluation, which included general examination, radiological examination and endoscopy of the nasal and paranasal sinuses. A detailed history, which included the age and sex, presence of predisposing factors such as long-term chemotherapy, immunosuppression and history of any chronic disease (diabetes, malignancy and others), was obtained for each patient.

The specimens for the diagnosis of FRS included allergic mucin, exudate from the nasal and paranasal mucosa and tissue biopsy from nasal polyp. Specimens were collected in sterile, screw-capped containers with labels that included pertinent patient information and sent immediately to the mycology laboratory. All the specimens received were processed by standard methods for fungal culture. The tissue specimens, after mincing into pieces, were subjected to initial screening by 10% potassium hydroxide (KOH) using light microscopy to look for fungal elements (septate or aseptate, hyaline or dematiaceous). Rest of the specimen was inoculated in duplicate onto Sabouraud dextrose agar with gentamicin and incubated at 25°C and 37°C for 4 weeks or until culture positive whichever was earlier. This was followed by slide culture and other special techniques wherever necessary. After slide culture, lactophenol cotton blue (LPCB) mounts was prepared to examine detailed morphological features of the fungi grown on the culture medium. Histopathological examination (HPE) was carried out for the tissue biopsy specimens. Haematoxylin and eosin (H and E) staining as well as Grocott-Gomori's methenamine silver (GMS) staining was performed. Histopathological confirmation of tissue invasion by the fungi is essential for classifying the FRS as invasive. Invasive FRS is defined as sinusitis supported by radiological findings and histopathological evidence of hyphal forms within sinus mucosa, submucosa, blood vessels or bone.^[4] A proper histopathological categorisation of FRS is important for optimal treatment.

Result

During the study period of 1 year (April 2009 to March 2010), specimens from the nose and paranasal sinuses of 60 clinically diagnosed cases of rhinosinusitis were sent for microbiological and HPE. All these specimens were from patients attending the OPD of the E.N.T. Department of SRMC and RI. Out of the 60 specimens, 45 showed no growth after 4 weeks of incubation. Specimens from the rest 15 cases (25%) grew various fungal isolates in culture. Out of these 15 cases, 9 were males and 6 females (M:F ratio 1.5:1). The age range was from 26 years to 84 years (average age, 54 years). 4 of the 15 patients were immunocompromised (3 diabetic, 1 malignancy). The duration of symptoms ranged from 2 weeks to 8 months. The chief complaints in these 15 cases included nasal discharge (81%), nasal obstruction (78%), headache (71%) and loss of

smell (68%). Fungal culture revealed 9 cases of *Aspergillus flavus*, 1 each of *Aspergillus fumigatus* and *Penicillium* species. The rest four specimens grew unusual fungal isolates, i.e. *Acremonium* sp., *Scedosporium apiospermum*, *Cladosporium cladosporioides* and *Lasiodiplodia theobromae*.^[10] *A. flavus* was the most common cause of FRS in the study group (60%, i.e., 9 out of 15). Unusual fungal isolates constituted 26.67% of all cases of FRS. HPE of the specimens revealed that 8 of the 15 cases had fungal ball (6 *A. flavus*, 1 *A. fumigatus* and 1 *Penicillium* species). HPE also revealed two cases of AFRS, both due to *A. flavus*. The rest five cases were of invasive fungal sinusitis. Computed tomography (CT) scan findings were positive in 13 of the 15 cases, which showed soft-tissue density, opacification in one or multiple sinuses. Features of bone destruction were seen in five cases. The case of FRS due to *L. theobromae* has been published.^[10] The rest three unusual causes of FRS are discussed below.

The first patient was a 50-year-old male who presented with nasal block on both sides, intermittent bleeding from the nose and anosmia on and off for 3 weeks. Patient was apparently normal 1 month back. The general physical examination was normal. Local examination of the nose showed multiple polyps in both middle meatus with mucoid discharge, but no paranasal sinus tenderness. CT scan of paranasal sinus showed soft-tissue density in bilateral frontal, ethmoidal, maxillary and sphenoid sinus. A clinical diagnosis of sinonasal polyposis was made. Functional endoscopic sinus surgery (FESS) with polypectomy was done under general anaesthesia. FESS is a safe surgical procedure used for the treatment of chronic rhinosinusitis.^[11] The tissue material from the polyp of left maxillary sinus was sent for histopathological and microbiological investigation.

H and E stained tissue sections showed polypoidal fragments of the nasal mucosa infiltrated by the large number of acute inflammatory cells [Figure 1a]. Focal granulomas with giant cell reactions were seen [Figure 1b]. On staining with GMS, narrow pieces of fungal hyphae were found occasionally [Figure 1c].

The minced tissue was subjected to 10% KOH mount, which revealed narrow septate hyphae. Fungal culture was done following standard methods. The colonies were slow growing, compact, moist at first suede like and white in colour, which turned to light pink with age [Figure 2a]. Microscopic examination after LPCB staining showed long awl-shaped phialides producing cylindrical, one celled conidia mostly aggregated in slimy heads at the apex of each phialide [Figure 2b]. From the above characteristics, the fungus was identified as *Acremonium* species. After FESS, the patient was put on antifungal medication (tablet itraconazole 100 mg orally twice a day) for 2 months and he recovered. Follow-up endoscopy revealed no recurrent disease.

The second case was that of a 67-year-old lady who presented with rhinorrhoea and post-nasal discharge for past 3 months and extra-nasal symptoms like numbness over the left half of face for 8 months, diplopia for 5 months, loss of vision in the right eye for 3 months and drooping of eyelids of 15 days duration. The patient was on regular treatment for immunoglobulin (Ig) A nephropathy. She was also a known case of hypertension and hypothyroidism on treatment. Physical examination showed that extra ocular movements on the left side were affected. Fundus could not be visualised. Bone conduction was greater than air conduction on the left side. Webers was lateralised to left. Conductive deafness was present. CT scan revealed a soft-tissue mass in the left orbit and thickening of the left medial rectus muscle. A clinical diagnosis of sinusitis was made. Endoscopic biopsy of left pterygopalatine fossa was done and sent for histopathological examination. Tissue sample from orbital apex was also sent for fungal culture and histopathology.

Histological features of biopsy specimen from left pterygopalatine fossa were suggestive of well-differentiated squamous cell carcinoma in left pterygopalatine fossa and infiltrating orbital apex. H and E stain of sections from the tissue sample of orbital apex showed small fragments of fibrocollagenous connective tissue with fragments of bone and eosinophilic homogeneous substance appearing like keratinous flakes with few chronic inflammatory cells [Figure 3a]. GMS staining of the tissue sample showed few septate hyphal fragments [Figure 3b].

10% KOH preparation of the tissue sample from the orbital apex revealed a few narrow septate hyphae. On culture, the colonies were fast growing, greyish white suede like with a greyish black reverse [Figure 4a]. Microscopic examination after LPCB staining showed numerous single celled, pale brown, broadly clavate to ovoid conidia with truncated bases. The conidia were borne singly, graphium state was also seen [Figure 4b, inset]. The fungus was identified as *S. apiospermum*, which is an anamorph of *Pseudallescheria boydii*. A final diagnosis of squamous cell carcinoma in left pterygopalatine fossa with fungal infection in orbit by

S. apiospermum was made. Patient was discharged on request and was advised to review urgently in an oncology centre.

The third patient was a 28-year-old male who presented with nasal block, dryness of the throat and nasal discharge since 2 months and headache for 1 month. The general physical examination was normal. Local examination of the nose showed left inferior turbinate hypertrophy with edematous mucosa. Antrochoanal polyps were present and there was no paranasal sinus tenderness. CT scan showed polyp occupying the right maxillary sinus, ethmoidal air cells and sphenoid sinus (pansinusitis). A clinical diagnosis of antrochoanal polyp was made. FESS with polypectomy was done under general anaesthesia. Tissue from the polyp was sent for histopathological and microbiological investigations.

HPE of the polyps revealed features suggestive of inflammatory polyp with large areas of necrosis, old haemorrhage and dystrophic calcification on H and E staining [Figure 5a], whereas GMS staining of the tissue showed entangled septate fungal hyphae [Figure 5b].

The minced tissue specimen was subjected to 10% KOH mount, which showed narrow hyphae with septations. On culture, the colonies were expanding, velvety to powdery, olivaceous green to olivaceous brown with reverse olivaceous black [Figure 6a]. Microscopic examination after LPCB staining showed conidiophores of variable length, without swellings, with terminal and lateral ramifications, bearing branched conidial chains, pale olivaceous brown. Conidia were ellipsoidal to limoniform, smooth walled or slightly verrucose, olivaceous brown, one celled, with dark scars, easily liberated [Figure 6b]. The fungus was identified as *C. cladosporioides*. For confirmation of identification, the fungal isolate was sent to Mycology Division, Post Graduate Institute of Medical Education and Research, Chandigarh. The isolate was confirmed as *C. cladosporioides* (NCCPF No.: 350015). Post-FESS, the patient was given tablet itraconazole 100 mg twice daily for 3 months. No further treatment was necessary and follow-up endoscopic examination showed clean sinuses.

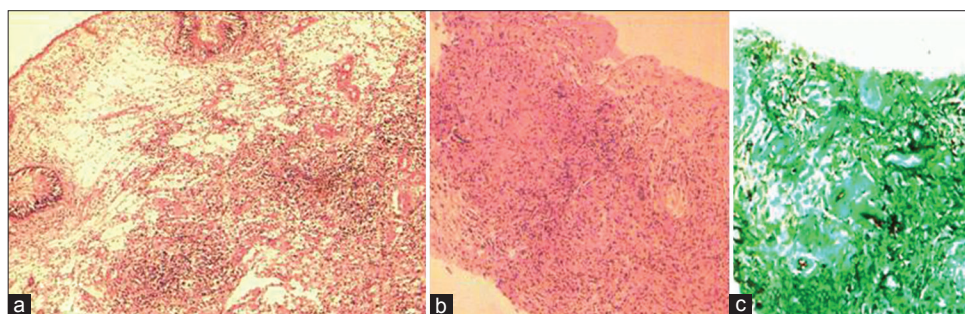


Figure 1: (a) H and E stained tissue section showing polypoid fragments of the nasal mucosa infiltrated by the large number of acute inflammatory cells (×400). (b) H and E stain showing focal granulomas with giant cell reactions (×400). (c) Grocott-Gomori's methenamine silver stain showing occasional slender septate fungal hyphae (×400)

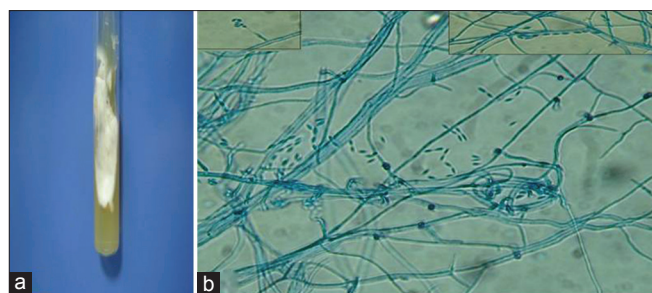


Figure 2: (a) Colonies slow growing, compact, moist at first suede like and white in colour which turned to light pink with age. (b) Hyaline, erect phiallides mostly arising singly from creeping hyphae and gradually tapering towards apex, producing cylindrical, one celled conidia mostly aggregated in slimy heads at the apex of each phiallide seen ($\times 100$)

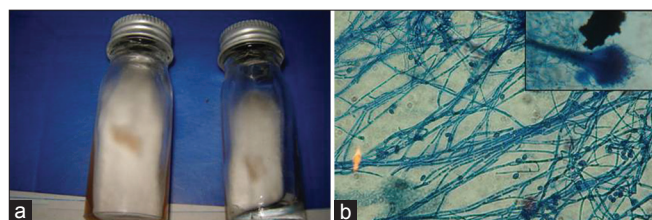


Figure 4: (a) Colonies fast growing, greyish white suede like with greyish black reverse. (b) Numerous single celled, pale brown, broadly clavate to ovoid conidia with truncated bases were seen. The conidia were borne singly ($\times 100$). Inset shows the graphium stage ($\times 400$)

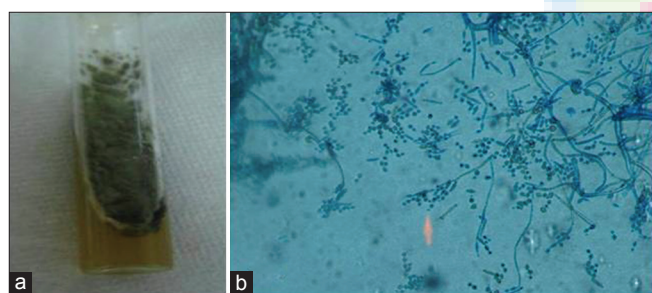


Figure 6: (a) Colonies expanding, velvety to powdery olivaceous green to olivaceous brown with reverse olivaceous black. (b) Conidiophores of variable length, without swellings, with terminal and lateral ramifications, bearing branched conidial chains. Conidia ellipsoidal to limoniform, 1 celled, with dark scars ($\times 100$)

Discussion

The aim of this study was to find out the occurrence of FRS caused by unusual fungal isolates in a tertiary care teaching hospital in South India. *A. flavus* has been reported to be the most common fungal isolate in studies on FRS from India.^[1,2] A recent study from the United States (US) by Montone *et al.*, have also found *Aspergillus* sp to be the most common cause of FRS.^[12] Other common causes of FRS in India include *A. fumigatus*, *Aspergillus niger*, *Rhizopus* spp etc.^[1,2,13] In our study also, we found *A. flavus* to be the most common cause of FRS (60%). However, reports of unusual causes of FRS have been on the rise from India as well as other countries.^[14] Baradkar *et al.*

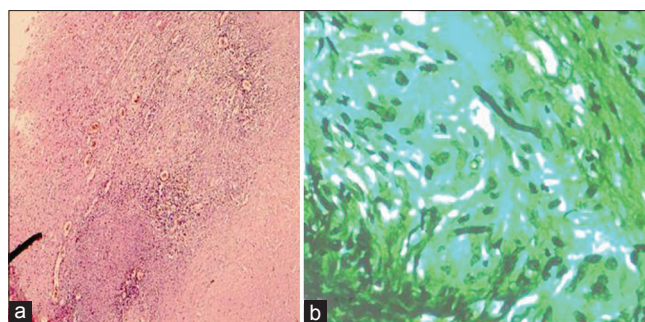


Figure 3: (a) H and E stained sections from tissue samples of orbital apex showing small fragments of fibrocollagenous connective tissue with fragments of bone and necrotic tissue with chronic inflammatory cells. Filamentous fungal elements with septations seen in necrotic debris ($\times 100$). (b) Grocott-Gomori's methenamine stain showing occasional septate hyphae ($\times 200$)

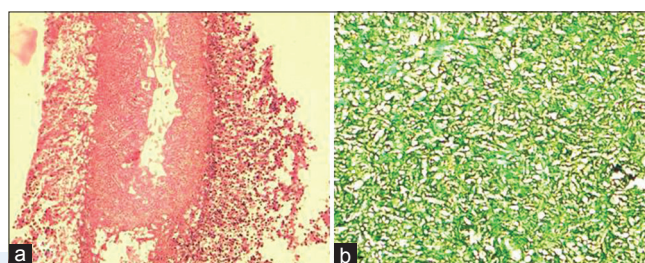


Figure 5: (a) H and E stained tissue section showing separate fungal ball with entangled hyphal forms ($\times 400$). (b) Grocott-Gomori's methenamine stain showing entangled fungal hyphae ($\times 400$)

have reported a fatal case of rhino-orbito-cerebral infection caused by *Saksenaea vasiformis* in an immunocompetent individual.^[5] In a case report by Swain *et al.*, *Schizophyllum commune* was reported to cause sinusitis in an immunocompetent individual.^[6] Another case reported from South India by Premamalini *et al.*; also found *S. commune* to be a causative agent of FRS.^[15] Janagond *et al.* reported a case of rhinosinusitis caused by *Trichosporon inkin* from South India.^[7] Shivaprakash *et al.* reported a case of AFRS caused by *Neosartorya hiratsukae* for the first time from India.^[16] In our study, we found unusual causes of fungi causing rhinosinusitis in 4 (26.67%) out of the 15 culture positive cases of FRS. The case of *L. theobromae* causing FRS has already been reported.^[10] Out of the rest three cases, there were no clear-cut predisposing factors, which could account for susceptibility to fungal infections in two of these patients (cases 1 and 3). The isolates in these two cases were *Acremonium* sp. and *C. cladosporioides*, which have been reported as causative agents of FRS from India and other countries also.^[2,8,9,17,18] Another case (patient 2) was a 67-year-old lady who was on regular treatment for IgA nephropathy and was diagnosed as having malignancy (squamous cell carcinoma of the left pterygopalatine fossa) at SRMC and RI. So, these underlying medical conditions might have made her immunocompromised and hence susceptible to fungal infections. Here, the fungus isolated was

S. apiospermum which can cause invasive fungal sinusitis in immunocompromised patients^[19] and is found to be refractory to therapy in some studies.^[20] In a recent study from US by Montone *et al.*, *S. apiospermum* was found to be a cause of chronic invasive FRS.^[12]

The cases of FRS are classified based on the histopathological features into invasive and non-invasive types.^[3] Acute invasive FRS is when the duration of illness is <1 month, whereas chronic invasive FRS has duration >3 months, along with other factors like immune status and vascular invasion, which may also differentiate between the two forms of FRS. In addition, acute FRS shows a neutrophilic tissue reaction while in chronic FRS, an eosinophilic reaction is usually seen.^[3,4] A new term subacute may be used in rare cases when the duration of illness is in between 1 and 3 months and a mixed cellular reaction is seen (both neutrophilic and eosinophilic).^[3] Granulomatous invasive FRS has time course of >12 weeks and histopathologically a granulomatous response is seen with fibrosis.^[3,4] Localised fungal colonisation of the nasal and paranasal sinus mucosa is the asymptomatic colonisation of mucous crusts within the nasal cavity in patients who have a history of sinus surgery and is detected upon endoscopic examination.^[3,4] Fungal ball is the noninvasive accumulation of dense conglomeration of fungal hyphae in one sinus cavity, or rarely multiple sinuses.^[3] In eosinophil related FRS including AFRS, it is believed that the fungal allergens elicit IgE-mediated allergic and type III (immune complex)-mediated mucosal inflammation. There is generalised inflammation of the sinonasal mucosa and viscid allergic mucin is present, which obstructs the normal drainage pathway. Fungi are present locally and stimulate locally destructive immune responses.^[3]

In all the three cases, HPE of the tissue biopsy material showed fungal elements in the tissue, which categorises all the three cases as invasive FRS.^[3,21] In the first case, the duration of illness was less than 1 month and HPE of the tissue biopsy material showed acute inflammatory cells and focal granulomas with giant cell reactions. The presence of granulomatous reaction causes the dilemma in classifying this case as acute invasive FRS. This case can better be defined as mixed pattern reaction where we find acute invasive FRS in addition to granulomatous reaction.^[4,21] In the second case, the duration of illness was more than 3 months and HPE of tissue samples from orbital apex showed eosinophilic homogeneous substance with few chronic inflammatory cells. These features are suggestive of chronic invasive FRS.^[3,21] In the third case, the duration of illness was 2 months, which suggests that this case might be considered as a sub-acute type of invasive FRS.^[3] Hence, along with the unusual causes of fungi causing FRS, these 3 cases re-emphasize the dilemma in the classification of FRS. Presently, there are more questions than answers regarding the categorisation of FRS.^[3,4]

FESS with polypectomy was done in two of the cases followed by anti-fungal therapy with oral itraconazole. A combined surgical and medical approach is recommended for the treatment of FRS.^[22] Oral itraconazole has been found to be beneficial in recalcitrant fungal sinusitis.^[23]

To conclude, unusual fungal pathogens causing infections of the nose and paranasal sinuses have been reported from some parts of India. Such infections are also found in South India as emphasized in our study. Hence, the clinicians, microbiologists and pathologists in this part of the country should be aware of such rare fungal pathogens for proper diagnosis and treatment.

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