Fungal Rhinosinusitis: Report of uncommon *Aspergillus* species as etiological agents

Chadiesh Nagarajan, Premamalini Thayanidhi, Anupma J Kindo, Vijayakumar Ramaraj, Sanjeev Mohanty, Ravikumar Arunachalam

ABSTRACT

Introduction: Rhinosinusitis caused by the usual *Aspergillus* spp. is very common. However, rare isolates of *Aspergillus* causing fungal sinusitis is also on the rise.

Case Series: We hereby report three cases of fungal sinusitis caused by uncommon *Aspergillus* such as *A. versicolor* and *A. sydowii*. Case 1 was a 40-year-old female came with complaints of nasal block and nasal discharge for past one and a half years with history of previous nasal surgery. Her computed tomography (CT) scan of paranasal sinus (PNS) showed bilateral ethmoidal sinusitis. Case 2 was a 43-year-old male known asthmatic presented with complaints of nasal block for last five years, was diagnosed to have bilateral sinonasal polyposis by anterior rhinoscopy. Case 3 was a 17-year-old female known asthmatic presented with headache, nasal discharge and frequent sneezing for last six months. Her CT PNS showed left side deviated nasal septum with left side pan sinusitis along with right frontal sinusitis. All the three patients underwent functional endoscopic sinus surgery (FESS). The material was sent to the microbiology laboratory for fungal culture and potassium hydroxide mount. Speciation by slide culture was not conclusive. Hence, molecular methods were opted for speciation.

Conclusion: Reporting of these cases will ensure awareness among the microbiologists about the not so common *Aspergilli* as a cause of fungal sinusitis. The need of molecular methods for speciation has also been emphasized here as it is difficult to speciate these *Aspergilli* using routine conventional methods.

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Introduction: Rhinosinusitis caused by the usual *Aspergillus* spp. is very common. However, rare isolates of *Aspergillus* causing fungal sinusitis is also on the rise. **Case Series:** We hereby report three cases of fungal sinusitis caused by uncommon *Aspergillus* such as *A. versicolor* and *A. sydowii*. **Case 1** was a 40-year-old female came with complaints of nasal block and nasal discharge for past one and a half years with history of previous nasal surgery. Her computed tomography (CT) scan of paranasal sinus (PNS) showed bilateral ethmoidal sinusitis. **Case 2** was a 43-year-old male known asthmatic presented

with complaints of nasal block for last five years, was diagnosed to have bilateral sinonasal polyposis by anterior rhinoscopy. **Case 3** was a 17-year-old female known asthmatic presented with headache, nasal discharge and frequent sneezing for last six months. Her CT PNS showed left side deviated nasal septum with left side pansinusitis along with right frontal sinusitis. All the three patients underwent functional endoscopic sinus surgery (FESS). The material was sent to the microbiology laboratory for fungal culture and potassium hydroxide mount. Speciation by slide culture was not conclusive. Hence, molecular methods were opted for speciation. **Conclusion:** Reporting of these cases will ensure awareness among the microbiologists about the not so common *Aspergilli* as a cause of fungal sinusitis. The need of molecular methods for speciation has also been emphasized here as it is difficult to speciate these *Aspergilli* using routine conventional methods. .

Keywords: *Aspergillus versicolor*, *Aspergillus sydowii*, Fungal Rhinosinusitis

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INTRODUCTION

Rhinosinusitis is inflammation of the nasal and paranasal sinus mucosa and is associated with mucosal

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alterations ranging from inflammatory thickening to gross nasal polyp formation [1]. *Aspergillus* spp. are saprophytes which are commonly present in the environment. Different species of *Aspergillus* can cause allergic, non-invasive and chronic invasive fungal sinusitis [2].

Aspergillus spp. is the most common fungal infection of the paranasal sinuses. The causative organism is a spore-forming filamentous fungus which occurs as a saprophyte in soil and decaying vegetable matter and is spread by airborne transmission. Transmission between humans is unknown [3]. The most common septate fungi causing fungal rhinosinusitis are *A. fumigatus* and *A. flavus*. In most parts of the world, the organism usually isolated is *A. fumigatus*. In India, *Aspergillus flavus* is isolated in more than 80% of the cases of acute fungal rhinosinusitis [4].

Rare isolates of *Aspergillus* causing fungal sinusitis is also being frequently reported as case studies all over India [4]. The findings from the present case indicate that it is sometimes difficult to conventionally identify the not so common *Aspergillus* spp. and molecular techniques should be opted for the correct identification.

CASE SERIES

Case 1: A 40-year-old female was presented with complaints of right nasal block and watery discharge for the past one and a half years. The patient had undergone a nasal surgery, three years before in another hospital. The exact details of the surgery could not be elicited from the patient. The computed tomography (CT) scan of paranasal sinus (PNS) showed bilateral ethmoidal haziness suggestive of a bilateral ethmoidal sinusitis. Functional endoscopic sinus surgery (FESS) was done. The material was sent to the microbiology laboratory for fungal culture. Potassium hydroxide mount showed hyaline septate hyphae. After a week, the fungal culture on Czapek-Dox agar grew yellow velvety colonies, on further incubation the culture changed its colour to yellow-green (Figure 1). The lactophenol cotton blue mount (LPCB) from slide culture, showed septate hyphae with acute angled branching. Conidial heads radiate, vesicles sub-spherical to ellipsoidal, conidiogenous cells biserial with phialides longer than or as long as metula (Figure 2). The isolate was identified as *Aspergillus* spp. The patient was then started on itraconazole 200 mg twice daily for six weeks. Since the isolate could not be identified up to the species level based on the microscopic morphology, it was taken for molecular identification with polymerase chain reaction (PCR) and gene sequencing. The culture grown on Sabouraud's dextrose agar was used for DNA extraction using the Qiagen kit as per manufacturer instructions. The internal transcribed spacer (ITS) regions (ITS1-5.8S-ITS2) was amplified using ITS1 (50-TCCGTAGGTGAACCTGCGG-3)

and ITS4 (50-TCCTCCGCTTATTGATATGC-30) as described previously [5]. The amplicon was sent for gene sequencing. The sequence was then used for a nucleotide - nucleotide search using the BLAST algorithm at the NCBI website (<http://www.ncbi.nlm.nih.gov/BLAST/>). BLAST hits more than 98% were considered. The identity was with *Aspergillus versicolor*. The isolate was identified as *Aspergillus versicolor*. The sequence was deposited in Genbank and the accession number is KC618327. Alignment of ITS1-5.8S-ITS2 regions of *Aspergillus versicolor* illustrating the sequence characteristics is shown in Figure 3. The patient was continued on

Figure 1: Yellowish to yellow-green colonies on Czapek-Dox agar.

Figure 2: Lactophenol cotton blue mount conidial head radiate, vesicle sub-spherical to ellipsoidal, conidiogenous cells biserial with phialides longer than or as long as metula.

the antifungal therapy with Itraconazole, she became asymptomatic and was discharged.

Case 2: A 43-year-old male was presented with complaints of frequent nasal block. He is a known asthmatic for the last 5 years on steroids. Using anterior rhinoscopy the patient was diagnosed of having bilateral sinonasal polyposis. The patient underwent FESS with polypectomy. The material was sent for fungal culture. Potassium hydroxide mount showed hyaline septate hyphae. Histopathological examination of the tissue showed septate hyphae with granulomatous inflammation. The patient was started on voriconazole 200 mg twice daily for 3 weeks. The fungal culture grew yellow velvety colonies, which on further incubation changed its colour to yellow-green on Czapek-Dox agar. The LPCB mount showed septate hyphae with acute angled branching. Based on the microscopic morphology the culture was identified as *Aspergillus* spp. The isolate was taken for molecular identification with PCR, Gene sequencing and BLAST algorithm as mentioned in Case 1 and was identified as *Aspergillus versicolor*. The sequence was deposited in Genbank and the accession number is KC618328. Alignment of ITS1-5.8S-ITS2 regions of *Aspergillus versicolor* illustrating the sequence characteristics is shown in (Figure 3). Patient's condition improved following treatment and was discharged.

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gi|480359935|gb|KC618327.1|-----ACCG
gi|480359936|gb|KC618328.1|
gi|471470697|gb|KC427092.1|AGGATCATTACTGAGTGGGGCTGCTCCGGGCGCCAACTCCCAACCG

gi|480359935|gb|KC618327.1|TGAATACCTAACACTGTGCTTCGGCGGGGAACCCCTCGGGGCGAGCC
gi|480359936|gb|KC618328.1|-----AGCCCTCTCTGGGCGAGCC
gi|471470697|gb|KC427092.1|TGAATACCTAACACTGTGCTTCGGCGGGGAACCCCTCGGGGCGAGCC
* * * * *

gi|480359935|gb|KC618327.1|GCCGGGACTAT--GACTTCATGC-TGAGGTGATGAGTCTGAGTCTGA
gi|480359936|gb|KC618328.1|GCCGGGACTACTGAACCTCATGCTGAGAGTGAAGTCTGAGTCTGA
gi|471470697|gb|KC427092.1|GCCGGGACTACTGAACCTCATGCTGAGAGTGAAGTCTGAGTCTGA
*****

gi|480359935|gb|KC618327.1|ATATAAATCAGTCAAACTTTCAACAATGGATCTCTGGTTCGGGCATC
gi|480359936|gb|KC618328.1|ATATAAATCAGTCAAACTTTCAACAATGGATCTCTGGTTCGGGCATC
gi|471470697|gb|KC427092.1|ATATAAATCAGTCAAACTTTCAACAATGGATCTCTGGTTCGGGCATC
*****

gi|480359935|gb|KC618327.1|GATGAAGAACGCGAAGTCCGATAAGTAAATGTAATTGCAGAAATCAG
gi|480359936|gb|KC618328.1|GATGAAGAACGCGAAGTCCGATAAGTAAATGTAATTGCAGAAATCAG
gi|471470697|gb|KC427092.1|GATGAAGAACGCGAAGTCCGATAAGTAAATGTAATTGCAGAAATCAG
*****

gi|480359935|gb|KC618327.1|TGAATCATGAGTCTTTGAACGACATTGGGCCCTGGCATTCGGGGG
gi|480359936|gb|KC618328.1|TGAATCATGAGTCTTTGAACGACATTGGGCCCTGGCATTCGGGGG
gi|471470697|gb|KC427092.1|TGAATCATGAGTCTTTGAACGACATTGGGCCCTGGCATTCGGGGG
*****

gi|480359935|gb|KC618327.1|GCATGCCTGTCGAGCGTATTGCTGCCATCAAGCCGCGCTTGTGTGT
gi|480359936|gb|KC618328.1|GCATGCCTGTCGAGCGTATTGCTGCCATCAAGCCGCGCTTGTGTGT
gi|471470697|gb|KC427092.1|GCATGCCTGTCGAGCGTATTGCTGCCATCAAGCCGCGCTTGTGTGT
*****

gi|480359935|gb|KC618327.1|GGGTCGTCTCCCCCGGGGGGACGGGCCCCGAAAGGCAGCGGGCGCACCG
gi|480359936|gb|KC618328.1|GGGTCGTCTCCCCCGGGGGGACGGGCCCCGAAAGGCAGCGGGCGCACCG
gi|471470697|gb|KC427092.1|GGGTCGTCTCCCCCGGGGGGACGGGCCCCGAAAGGCAGCGGGCGCACCG
*****

gi|480359935|gb|KC618327.1|TGTCGGTCTCTGAGCGTATGGGGCTTTGTCAACCCGCTCGACT-AGGGCC
gi|480359936|gb|KC618328.1|TGTCGGTCTCTGAGCGTATGGGGCTTTGTCAACCCGCTCGACT-AGGGCC
gi|471470697|gb|KC427092.1|TGTCGGTCTCTGAGCGTATGGGGCTTTGTCAACCCGCTCGACT-AGGGCC
*****

gi|480359935|gb|KC618327.1|GGCCGGGCGCCAGCGACGTCTCAACCATTTTCTTCAGGT-GACCTCG
gi|480359936|gb|KC618328.1|GGCCGGGCGCCAGCGACGTCTCAACCATTTTCTTCAGGTGGAATCG
gi|471470697|gb|KC427092.1|GGCCGGGCGCCAGCGACGTCTCAACCATTTTCTTCAGGT-GACCTCG
* * * * *

gi|480359935|gb|KC618327.1|GATCAGCC--GAGGGGGGATCCC--GMGA
gi|480359936|gb|KC618328.1|GAGGA-GTCTTGCATGGGGAGCCTCCGGGAGA
gi|471470697|gb|KC427092.1|GATCATTCTCGACGGGACCG--CG--GAGA
* * * * *

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Figure 3: Alignment of ITS1-5.8S-ITS2 regions of *Aspergillus versicolor* (KC618327 and KC618328) and *Aspergillus sydowii* (KC427092) illustrating the sequence characteristics. (*) indicates identical nucleotides, (-) indicates gap introduced for alignment.

Case 3: A 17-year-old female presented with complaints of headache, nasal discharge and recurrent sneezing on and off for the last six months. Patient is a known case of bronchial asthma for the last three months. The CT PNS showed left pan sinusitis with right frontal sinusitis with deviated nasal septum to the left side. FESS with septoplasty was done and the material was sent for culture. The potassium hydroxide mount showed septate hyphae. The patient was started on itraconazole 200 mg twice daily for six weeks. Fungal culture grew spreading blue-green colonies with reddish reverse on Czapek-Dox agar (Figure 4A-B). The LPCB mount showed radiate conidial heads, smooth walled conidiophores, vesicles spherical to sub-spherical, conidiogenous cells biserial (Figure 5). Since there was a difficulty in identifying the *Aspergillus* up to species level, the isolate was taken for PCR, Gene sequencing and BLAST algorithm as mentioned in the Case 1 and was identified as *Aspergillus*

Figure 4: (A and B) Colonies on Czapek-Dox agar showing spreading, blue-green, with straw colored to reddish brown shades with reddish reverse.

Figure 5: Lactophenol cotton blue mount showing radiate conidial heads, vesicles spherical to sub-spherical, conidiogenous cells biserial.

sydowii. The sequence was deposited in Genbank and the accession number is KC427092. Alignment of ITS1-5.8S-ITS2 regions of *Aspergillus sydowii* illustrating the sequence characteristics are shown in (Figure 3). The patient improved and was discharged.

DISCUSSION

Like other species of *Aspergillus*, *A. versicolor* is ubiquitous in nature and can be isolated from soil, water, organic matter, bathrooms, carpets, and mattresses [6]. It is commonly found on water-damaged building material such as wallpaper or fiberboard insulation. *A. versicolor* infections in human beings have been more commonly associated with onychomycosis and pulmonary aspergillosis [7, 8] and other different human mycoses [9].

In 2012, Dnyaneshwari et al. reported a case of chronic fungal rhinosinusitis caused by *A. versicolor* in a 35-year-old immunocompetent female, who presented with a history of nasal block and change of voice [10].

Aspergillus sydowii is a saprophytic fungus found in soil that can contaminate food and is occasionally pathogenic to humans. *Aspergillus sydowii* has been implicated in the pathogenesis of several human diseases, including aspergillosis, onychomycosis and keratomycosis [9].

Fungal sinusitis which was uncommon initially is now being more frequently reported. *Aspergillus versicolor* and *Aspergillus sydowii* are rare causes of fungal sinusitis. Though these rarer isolates are less pathogenic, they may cause invasive disease in severely immunocompromised patients [11].

In our case series, the first patient was diagnosed as a case of chronic fungal rhinosinusitis (CFRS) caused by *Aspergillus versicolor* and the second patient was complicated by bilateral nasal polyps. The third patient was having CFRS caused by *Aspergillus sydowii*. The first two patients who had *A. versicolor* as the etiological agent were started on antifungal therapy with itraconazole and voriconazole, respectively. They improved clinically and were discharged. The third patient who grew *A. sydowii* on culture was started on itraconazole and patient clinical condition improved markedly.

Hedayati et al. in a study on CFRS, had demonstrated that fungi can be isolated from patients undergoing sinus surgery using standard mycology laboratory protocol which is inexpensive and readily available. But in our study, molecular methods were required to confirm the isolate up to the species level, since the isolate could not be identified by the routine conventional phenotypic methods.

CONCLUSION

In our study, the isolated *Aspergillus* spp. are infrequent human pathogens. As it is a rare cause,

further study and identification up to the molecular level is necessary. Early diagnosis is essential in order to avoid destructive disease and start appropriate treatment before the symptoms becomes irreversible.

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Author Contributions

Chadiesh Nagarajan – Acquisition of data, Analysis and interpretation of data, Revising it critically for important intellectual content, Final approval of the version to be published

Premamalini Thayanidhi – Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published

Anupma J Kindo – Substantial contributions to conception and design, Analysis and interpretation of data, Revising it critically for important intellectual content, Final approval of the version to be published

Vijayakumar Ramaraj – Analysis and interpretation of data, Revising it critically for important intellectual content, Final approval of the version to be published

Sanjeev Mohanty – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Revising it critically for important intellectual content, Final approval of the version to be published

Ravikumar Arunachalam – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Revising it critically for important intellectual content, Final approval of the version to be published

Guarantor

The corresponding author is the guarantor of submission.

Conflict of Interest

Authors declare no conflict of interest.

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