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CASE REPORT

Aspergillus oryzae Isolated from Lung Tissue Sample in a Chronic Respiratory Illness Patient from South India

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ABSTRACT

Opportunistic fungal infection by *Aspergillus oryzae* in patients with chronic respiratory condition is rare in India. A 52-year-old male with a history of diabetes mellitus, chronic smoking, and alcoholism presented with chronic cough, breathlessness, and recurrent blood in sputum. CT imaging revealed features consistent with old pulmonary tuberculosis sequelae and the presence of a right lung lower lobe aspergilloma. The patient underwent a right lower lobectomy, and histopathological examination confirmed severe acute and chronic bronchiolitis with bronchiectasis and organizing pneumonia, indicative of fungal ball formation. Microbiological analysis identified *Aspergillus species,* possibly *A. flavus* complex, with sequencing confirming *A. oryzae*. This case is unique as *A. oryzae* is typically associated with food fermentation and rarely implicated in invasive lung infections, especially outside regions of traditional exposure, like Japan. The rarity of this presentation underscores the importance of considering unusual pathogens in clinical practice and utilizing advanced diagnostic techniques such as sequencing for accurate identification. The successful outcome of this case, with prolonged antifungal therapy based on microbiological confirmation and clinical follow-up, suggests the importance of personalized treatment strategies. This case report adds to the growing body of evidence on the clinical significance and pathogenic potential of *A. oryzae*. It underscores the importance of continued vigilance and research in fungal infections.

KEYWORDS: Aspergilloma, diabetes mellitus, Koji mold, opportunistic fungal infections, sequencing, smoker

INTRODUCTION

Opportunistic fungal infections are caused by *Aspergillus spp.* in patients with chronic respiratory conditions, immunocompromised hosts like haematological malignancies, and transplant recipients. Pulmonary infections caused by *Aspergillus spp.* vary in clinical presentation, namely aspergilloma, chronic necrotizing pulmonary aspergillosis, invasive pulmonary aspergillosis and allergic bronchopulmonary aspergillosis.[1] The diagnostic criteria for chronic pulmonary aspergillosis (CPA) of the European Society for Clinical Microbiology and Infectious Diseases (ESCMID), the European Respiratory Society (ERS) and the European Confederation of Medical Mycology (ECMM) include

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(i) one or more cavities with or without a fungal ball or nodules present on thoracic imaging for ≥3 months, (ii) direct evidence of *Aspergillus* infection or an immunological response to *Aspergillus spp.,* and (iii) exclusion of alternative diagnoses. Diagnosing this condition with appropriate evidence is essential as the management duration is at least 6 to 12 months. Microbiological evidence is the key to confirming the *Aspergillus* infection, so direct microscopy for hyphae or fungal culture from respiratory secretions, such as broncho-alveolar lavage (BAL) sampling or tissue/lung biopsy and histology reports, is recommended.[2]

Most of the studies conducted on the speciation of *Aspergillus* causing CPA have shown that *A. fumigatus* is the most common cause, followed by *A. niger, A. flavus, A. nidulans, A. terreus* and other rare species.[3] *A. oryzae* is a fungus widely used in traditional fermentation industries, including soy sauce, sake, bean curd seasoning and vinegar production.[4] It is infrequent to isolate *A.oryzae* as it does not commonly appear as a human pathogen. Based on the review of the literature through the PubMed search engine, it was noticed that only very few case reports have been published in the past. *A.oryzae* has been documented as a causative pathogen in meningitis in a case of paranasal sinusitis and a patient with pulmonary aspergilloma.[5-7] As this fungal infection in humans is extremely rare, cautious reporting should be done with adequate evidence and appropriate microbiological investigations, preferably with sequencing reports. A close taxonomical association is seen with *A. flavus* and *A. oryzae* with genome similarity. Hence, morphological findings and sequencing reports confirm the species' pathogenicity in humans.

This report presents a rare case of pulmonary aspergilloma caused by *A. oryzae*, which was identified and confirmed using the sequencing technique.

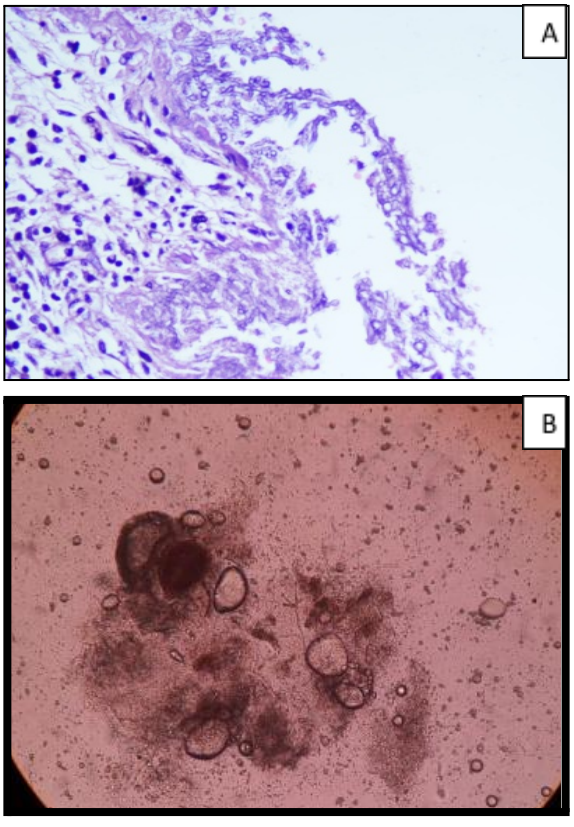
CASE REPORT

A 52-year-old male patient, chronic smoker and alcoholic, who had a known case of diabetes mellitus presented to the outpatient clinic in mid of 2023 with complaints of cough with expectorations on and off for the past five years, which aggravated over the last six months, breathlessness and recurrent episodes of blood in sputum since two months. The patient was diagnosed with laboratory-confirmed pulmonary tuberculosis (PT) in 2015 and completed anti-tubercular therapy.

Owing to the history of tuberculosis, the patient's sputum sample was sent for acid-fast bacilli (AFB) staining, which showed a negative finding. A sputum specimen was sent for Xpert MTB-RIF assay (Cepheid), which also showed negative results. In contrast, the computed tomography (CT) of the chest showed features suggestive of old PT, right lung lower lobe aspergilloma, and alveolar opacification in the upper and lower lobes of both lungs. A clinical diagnosis of old PT sequelae with suspicion of active fungal infection was made, and the patient was advised for the right lower lobectomy.

He underwent the procedure, and the postoperative period was uneventful. During the procedure, the right lower lobe mass was identified with a size of 5X5 cm^2 in the apical portion of the lower lobe. Hence, the right lower lobe was excised along the mass in toto. The excised tissue was sent for histopathological examination (HPE) and bacterial and fungal culture and susceptibility testing. The patient was treated with IV antibiotics - levofloxacin 750 mg once daily and oral itraconazole 200 mg twice daily for two weeks and followed up after 14 days with the culture and HPE report.

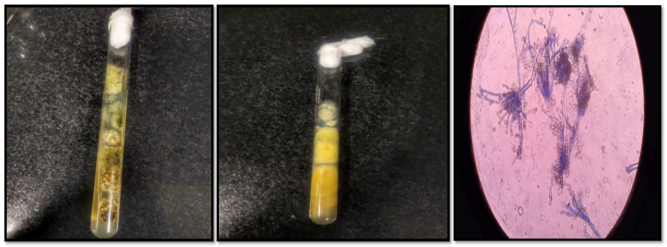
Figure 1: Histopathological imaging of the lung tissue biopsy sample. (A) *Microscopic sections studied from the lesion showed severe acute and chronic bronchiolitis with patchy fibrosis, consistent with bronchiectasis and organizing pneumonia; (B) Direct potassium hydroxide (KOH) mount of the tissue samples revealed few branching septate hyphae, suggestive of aspergillosis.*



Microscopic sections studied from the lesion showed severe acute and chronic bronchiolitis with patchy fibrosis, consistent with bronchiectasis and organizing pneumonia [Fig 1A]. Special staining of the sections revealed the presence of fungal organisms showing slender hyphae with acute angle branching, suggestive of aspergillosis [Fig 1B].

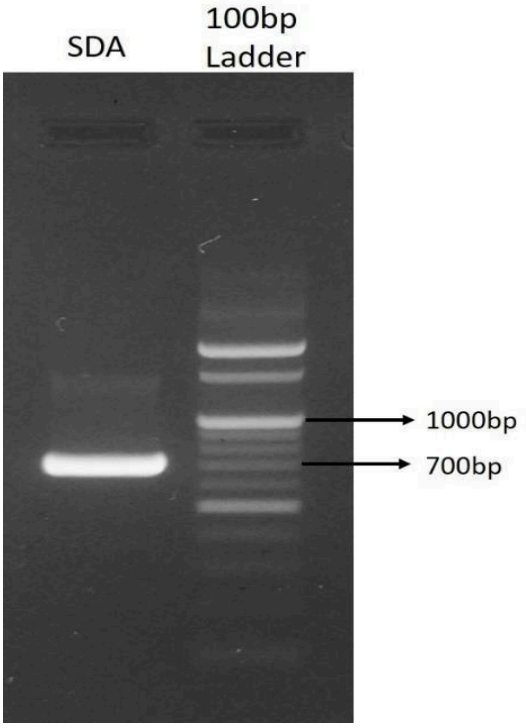
Aerobic bacterial culture and subculture from thioglycollate broth on MacConkey, blood agar and mannitol salt agar showed no growth. On Sabouraud's Dextrose Agar, colonies appeared on the second day that looked granular and flat, and the colony was initially white, then turned to yellowish green on the fourth day and then to greenish brown on subsequent days [Figure 2]. Microscopically, it revealed conidial heads that are globose or radiate with loose columns. The conidia were large, approximately 5-8 µm in diameter, typically radiate, later splitting to form loose columns and were globose, pale green and had biseriate arrangements, but some heads with phialides borne directly on the vesicle. The organism was identified as *an Aspergillus* species, possibly a *flavus* complex. Since the color of the colony turned slightly brown on SDA media, which may be seen in older cultures, and phialides were longer than usual *A. flavus* on Lactophenol Cotton Blue (LPCB) mount, we sent the isolate for sequencing to confirm whether it is *A. flavus* or some other *Aspergillus spp.*

Figure 2: Mycological culture in Sabouraud's Dextrose Agar and Microscopic features of the colonies. Colonies appeared on the second day, looking granular and flat. The colony was initially white and then turned yellowish-green on subsequent days. Microscopically, it revealed conidial heads that radiate with loose columns and were globose, pale green and had biseriate arrangements, but some heads with phialides borne directly on the vesicle.



Using universal primers, a polymerase chain reaction was performed per standard protocol to amplify the Internal transcribed spacer (ITS) gene. Universal ITS gene primers were selected from previous studies for complete amplification.[8] The amplified products were checked on 1.5% agarose gel electrophoresis, and the molecular weight was checked using a molecular weight marker (100bp ladder) [Figure 3]. The sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA). The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using MEGA 7.

Figure 3: PCR Gel electrophoresis. The image showed the amplified product that was checked on 1.5% agarose gel electrophoresis.



The similarity index built in the NCBI's BLAST program confirmed the amplified sequences belonging to the ITS gene. Based on the higher percentage similarity against the reference species, the species being investigated was assigned to *Aspergillus oryzae NRRL 447.*

Based on the histopathological and microbiological findings, oral itraconazole 200 mg twice daily was continued for 12 months. The patient is currently on follow-up as an outpatient with no complaints or complications.

DISCUSSION

We present a rare case of *Aspergillus oryzae* causing invasive fungal infection in the lungs, which presented as chronic invasive aspergillosis in a PT patient as sequelae. *A.oryzae*, called Koji Mold, has been

extensively used in Japan to produce sake, miso, and soy sauce for over 1000 years.[9] Very few case reports of *A.oryzae* are reported, mainly among those who worked in rice fields in Japan and those who are immunocompromised [Table 1]. In this case, we have noted that the patient belonged to South India and had no history of travelling to regions where this fungal entity is reported, which is a rarity in this region. Clinical diagnosis of aspergilloma helped in the early initiation of azoles. In laboratory diagnosis, the morphological findings from the lung tissue did not correlate with the common *Aspergillus spp.* It *was* doubtful that the microbiologist would confirm it with sequencing. Most centres do not have sequencing facilities or do not confirm the doubtful isolates with sequencing, especially in mycological samples, leading to underreporting of uncommon pathogens. This case report highlights the importance of sequencing uncommon isolates to confirm the pathogenicity of novel fungal pathogens and the probability of pathogenesis in existing filamentous saprophytic fungi like *A. oryzae*.

Table 1: Review of case reports on *Aspergillus oryzae* causing pulmonary infections.

Author	Year	Location	Clinical History	Treatment
Gordon et al.,[5]	1976	New York	*A. oryzae* meningitis	Amphotericin B and flucytosine.
Schwetz et al.,[12]	2007	Austria	A case of peritonitis caused by *A. oryzae* in a man on CAPD therapy	Amphotericin B and caspofungin, followed by itraconazole
Wilson et al.,[13]	2018	California	Chronic meningitis due to *A. oryzae*	Not given
Kume et al.,[14]	2022	Japan	Bronchial asthma due to *A. oryzae*	Mainly anti-allergics and bronchodilators

The CPAnet consensus statement published in 2018 stated that chronic pulmonary aspergillosis is a neglected fungal infection, and many fundamental questions need more collaborative research activities to address them.[10] Species identification is based on morphology, which is nowadays considered to discriminate the *Aspergillus* species insufficiently.[10] Accurate species-level identification of *Aspergillus* and maintaining a fungal registry is crucial as we look at azole-resistant fungi. In addition, antifungal testing criteria are not available for uncommon species, which is clinically challenging to correlate if the patient does not respond to the commonly used azoles. Hence, doubtful morphology requires confirmation with sequencing. IDSA recommends that antifungal therapy should be continued for 6-12 weeks.[11] However, prolonged therapy based on microbiological confirmation and clinical follow-up helped achieve a good prognosis in the above patient.

Furthermore, the identification of *Aspergillus oryzae* as a causative agent prompts a reevaluation of its clinical significance and pathogenic potential. This case emphasizes the need for continued surveillance and research into less conventional fungal pathogens, expanding our understanding of their ecological niches and potential impact on human health. The successful application of sequencing methodologies facilitates a precise diagnosis and enables a targeted and timely therapeutic intervention.

CONCLUSION

Ultimately, this case report contributes to the growing body of literature on fungal infections, urging clinicians and researchers to remain vigilant in their diagnostic approaches and treatment strategies, particularly as new insights into fungal diversity and pathogenicity continue to emerge through advanced molecular techniques. In addition, prompt diagnosis and appropriate antifungal therapy duration must be critically followed to ensure microbiological and clinical cure.

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INFORMED CONSENT

Written informed consent was obtained from the patient. Confidentiality of the patient was maintained in the article.

CONFLICTS OF INTEREST STATEMENT

The authors declare no conflict of interest.

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None

AUTHOR'S CONTRIBUTION

VM: Conceptualization, Microbiological analysis; Writing the draft
VG: Clinical Resources; Writing the draft
MM: Supervision; Validation; Review and editing

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