

***Tritirachium oryzae* causing pulmonary infection: A rare case report from India**

Renu Kumari Yadav; Immaculata Xess; Mragnayani Pandey; Bhaskar Rana; Gagandeep Singh*

***Corresponding Author: Gagandeep Singh**

Additional Professor, Department of Microbiology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110029, India.

Email: drgagandeep@gmail.com

Abstract

The first case of *Tritirachium oryzae* isolated from pleural fluid of an Indian patient is reported. A 71-year-old female patient who is a follow up case of carcinoma colon, presented with complaint of recurrent vomiting, pain abdomen and loss of appetite. A possibility of disease relapse was kept and evaluation done. Whole body PET CT revealed bilateral pleural effusion. Fungal culture of pleural fluid revealed growth of a pigmented mould after 5 days of incubation. It was identified as *Tritirachium oryzae* on the basis of gross morphological characteristics and microscopic characterization on slide cultures. The diagnosis of *T. oryzae* was confirmed by PCR sequencing of the internal transcribed region of rDNA using the primer pairs ITS4 and ITS5. In vitro antifungal susceptibility test was also performed.

Initially before the fungal culture result came patient was started on ATT as patient had a history of pulmonary tuberculosis and no other diagnosis was made. After the fungal culture report patient was planned to start on voriconazole but patient condition deteriorates and she succumbed.

Keywords: *Tritirachium spp.*, Pleural fluid; Fungal culture; AFST; PCR.

Introduction

The genus *Tritirachium* is known to cause infection in insects. The natural habitat of this genus is soil and decaying plant material. Human infection by *Tritirachium* is rare; only a few case reports exist. *Tritirachium* is reported from corneal ulcer, scalp, nail and ear infections.

We report the first case of pulmonary infection caused by *Tritirachium spp.* isolated from pleural fluid from a 71-year-old immunocompromised female patient.

Case Report

A 71-year-old female patient who is a follow up case of carcinoma colon, diagnosed in april 2018, Right hemicolectomy was done and patient was on chemotherapy. Patient also has a past history of pulmonary tuberculosis. Now patient presented with complaint of recurrent vomiting, pain abdomen and loss of appetite but passing flatus and stool. A possibility of disease relapse was kept and evaluation done, abdomen x-ray revealed multiple air fluid level; RT insertion with continuous drainage was done after surgical oncology opinion. Whole body PET CT revealed bilateral pleural effusion (L>R) and no other metabolically active lesion elsewhere. A diagnostic pleural tap was done and sent for AFB, gene xpert, amylase, sugar, ADA, malignant cytology, bacterial and fungal culture. All the investigations were negative and in mycology 10% potassium hydroxide preparation revealed no fungal elements but growth of a pigmented mould was seen on sabouraud dextrose agar with antibiotics after 5 days of incubation at 25 C. LPCB mount was prepared to identify the fungi.

Phenotypic Identification – Colonies on SDA showed vinaceous to lilac velvety surface growth on obverse and reverse was tan no pigmentation (Figure 1).

Slide culture preparations with LPCB stain showed subhyaline hyphae, which were pale to brown, smooth, and thin-walled. Conidiophores and conidiogenous cells were subhyaline. Conidiophores were well differentiated with thick-walled, suberect, and verticillately branched in the upper part of the conidiophores. Conidiogenous cells were arranged sympodially with strongly tapered and flask-shaped fertile rachis. Conidia were hyaline, smooth, and spherical to ellipsoidal. The isolate was identified morphologically as *Tritirachium oryzae* (Figure 2).

Confirmation of *T. oryzae* by Molecular Identification- Molecular identification was performed by sequencing the Internal Transcribed Sequence (ITS) region of rDNA using the primer pairs ITS4 and ITS5.

In vitro Antifungal Susceptibility Testing (AFST)- AFST was performed according to the CLSI standard; the results were- AmB 2.0 µg/ml, VOR 0.03 µg/ml, ITR 0.03 µg/ml, POS 0.03 µg/ml, CSP 0.12 µg/ml, and MFG 1.0 µg/ml.

Initially before the fungal culture result came patient was started on ATT as patient had a history of pulmonary tuberculosis and no other diagnosis was made. After 2-3 days of ATT her symptoms reduced and she started tolerating orally but suddenly her condition deteriorates. After the fungal culture report patient was planned to start on Voriconazole but patient condition deteriorate and she succumbed.

Table 1: Global review of *Tritirachium* human infections.

Agent	Site of infection	Country	Age (yrs)	Gender	Identification	Treatment	Year	References
<i>Tritirachium roseum</i>	Left eye corneal ulcer	USA	29	Male	Phenotypic	Nystatin, penetrating keratoplasty	1975	Rodrigues MM et al. [5]
<i>Tritirachium oryzae</i>	Otomycosis	India	45	Male	Phenotypic	Clotrimazole	2018	Sharma SK et al. [6]

<i>Tritirachium oryzae</i>	Finger nail	Iran	44	Female	Phenotypic and genotypic (ITS rDNA)	Oral itraconazole	2013	Naseri A et al. [7]
<i>Tritirachium oryzae</i>	Human scalp infection	Brazil	4	Female	Phenotypic	Topical treatment with ketoconazole shampoo	2010	Moraes RN et al. [8]
<i>Tritirachium oryzae</i>	DLSO great toe nail	India	22	Female	Phenotypic and genotypic (ITS rDNA)	Terbinafine, ketoconazole nail avulsion	2018	Vanam HP et al. [9]
<i>Tritirachium oryzae</i>	Pleural fluid	India	71	Female	Phenotypic and genotypic (ITS rDNA)	ATT, Voriconazole	2022	Present study



Figure 1: Growth of *Tritirachium* on Sabouraud Dextrose Agar.

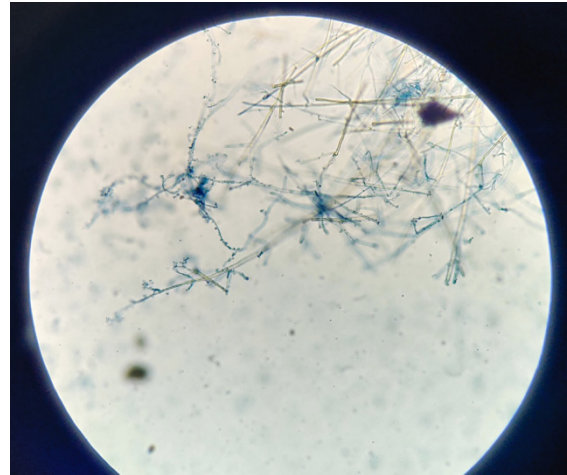


Figure 2: *Tritirachium* on Lacto phenol cotton blue mount (LPCB).

Discussion

The description of *Tritirachium* was first given by Limber in 1940 [1]. In India *Tritirachium* species was first described in 1967 [2]. Historically genus *Tritirachium* was placed in suborder Hyphomycetes which is obsolete now, and in recent taxonomical review, they are placed in subdivision Pezizomycotina. The new lineage Puccinomycotina includes class—Tritirachiomycetes, order—Tritirachiales, and family—Tritirachiaceae [3]. The genus *Tritirachium* has three accepted species: *T. dependens*, *T. oryzae*, and *T. cinna-momeum*. Other species which are under revision include *T. roseum*, *T. egeum*, and *T. album* [4]. Our isolate is matching with the description of *T. oryzae*. In this case of pleural effusion direct KOH mounts showed no fungal elements but grew readily on all the 3 tubes of SDA with cycloheximide, and gentamicin.

Though rare, human infections with *T. oryzae* have been reported in causing superficial mycoses which includes well-documented cases of corneal ulcers [5], otomycosis [6], onychomycosis [7], and scalp infection [8] but there have been no records of this fungus as the etiologic agent of pulmonary infection. In conclusion, *T. oryzae* is reported for the first time as the cause of pulmonary infection.

The paucity of isolates and fewer descriptions on *T. oryzae* bring out the succinct need for easy accessibility of molecular tools in making the identification less cumbersome. It is evident that *T. oryzae* is emerging as an opportunistic pathogen after its reported human cases. Future directions may include more studies on newer isolates and a robust antifungal agent reference spectrum. Molecular tools with classical description shall guide the trajectory of accurate taxonomical identification.

Declarations

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Authors Information: Renu Kumari Yadav; Immaculata Xess; Mragnayani Pandey; Bhaskar Rana; Gagandeep Singh*
Department of Microbiology, All India Institute of Medical Sciences, India.

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