International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Case Report

Isolation of *Exophiala Mesophila* from Respiratory Tract of an Immunocompromised Patient

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Received: 30/11/2014 *Revised: 11/02/2015* *Accepted: 02/03/2015*

ABSTRACT

Phaeohyphomycosis remains a loosely defined term designating infections caused by molds with dark walls in culture but not necessarily in tissue. The increasing incidence and prevalence of human infection owe to its interference with host immune mechanism by debilitating diseases, chemotherapy, and radiotherapy. Most opportunistic fungal infection is caused by exogenous fungi that exist in nature as either saprophyte or plant pathogen. Of all the agents of phaeohyphomycosis, infection with species of Genus *Exophiala* have been reported frequently from clinical and non clinical sources suggesting that this organism is widely and abundantly present in nature as saprophyte. Here we report a case where *Exophiala mesophila* was isolated from sputum of a patient of Type 2 Diabetes Mellitus and Pulmonary Tuberculosis. Molecular identification was done by sequencing the internal transcribed region (ITS) of rDNA including ITS1, 5.8S and ITS2. The patient was put on voriconazole 200 mg twice daily for 10 days. Following treatment, she showed improvement and follow up sputum culture after 3 months showed absence of growth of the fungus.

Key Words: *Exophiala mesophila;* Black yeast; Diabetes mellitus; respiratory tract; phaeohyphomycosis

INTRODUCTION

Phaeohyphomycosis is a term introduced by Ajello in 1974 for hyphomycetous fungi with darkly pigmented, septate hyphae. [1] It remains a loosely defined term designating infections caused by molds with dark walls in culture but not necessarily in tissue. The increasing incidence and prevalence of human infection owe to its interference with host immune mechanism by debilitating diseases, chemotherapy, and radiotherapy. Most opportunistic fungal infection is caused by exogenous fungi that exist in nature as either saprophyte or plant pathogen. It may involve the cutaneous, subcutaneous tissue, central nervous system or other internal organs such as liver, lungs and pancreas. Of all the agents of phaeohyphomycosis, infection with species of Genus *Exophiala* have been reported frequently from clinical and non clinical sources suggesting that this

organism is widely and abundantly present in nature as saprophyte. Though species under the genus *Exophiala* can be identified on the basis of conventional identification techniques like morphology and physiological characters, due to the diversity of the species that has been recently described within this Genus use of molecular methods is essential to confirm its identity. [2] The sequence diversity of the internal transcribed spacer regions, of rRNA has proven to be reliable for species distinction in the genus *Exophiala*. [3] Here we report a case where *Exophiala mesophila* was isolated from sputum of a patient of Type 2 Diabetes Mellitus and Pulmonary Tuberculosis.

CASE REPORT

A 73 years old diabetic female with complaints of severe cough of 3 months duration in respiratory distress was admitted in the Intensive Care Unit (ICU) of a tertiary care hospital in Assam. On admission, her fasting and postprandial blood sugar levels were 230 mg% and 302 mg% respectively. Other biochemical test reports were within normal limits. Chest X-ray showed features suggestive of bilateral lower zone interstitial pneumonitis (right > left). She was put on fluoroquinolones and insulin soluble (Human Actrapid) to which she responded. Three months later, she again developed severe cough with expectoration and high fever. Chest X-ray revealed left lower lobe consolidation. Subsequent CT scan of the thorax also showed patchy consolidation in the superior segment and posteromedial basal segment of the lower lobe of the left lung. Three consecutive sputum samples tested for acid fast bacilli (AFB) were negative. Sputum culture grew *Klebsiella spp*. and *Candida albicans*. She was treated with ciprofloxacin 500mg thrice a day and intravenous fluconazole 200mg/day for 7 days. Though she showed some improvement in terms of relief of symptoms, in between, she had regular bouts of cough and fever. The sputum at this point of time was blood stained and mucopurulent. Bronchoscopy was done under local anaesthesia and BAL fluid was taken from both lower lobar segmental bronchi. Qualitative PCR for *Mycobacteria tuberculosis* was positive. Cytology examination of BAL fluid was negative for malignant cells. She was then put on short term anti-tubercular therapy (Rifampicin 450mg, INH 300mg, Pyrazinamide 1500mg, Ethambutol 800mg X 2 months followed by rifampicin 450mg, INH 300mg, Ethambutol 800mg for 4 months). Though she had some relief in symptoms, she continued to have cough with mild fever. Her blood sugar levels fluctuated during this period. She developed another episode of severe pneumonia like features that required ICU admission. This time the sputum was also sent for fungal culture. KOH mount of sputum revealed pigmented fungal elements (Fig 1). The sample was plated on Sabouraud Dextrose Agar and Mycosel (Sabouraud Dextrose Agar with Chloramphenicol and Cycloheximide) and kept at room temperature. After 5 days, culture showed growth of small colonies that were dark brown and velvety on the obverse and black on the reverse (Fig: 2). Lactophenol cotton blue mount revealed septate, brown & branched hyphae. There were conidiophores with conidiogenous cells (Fig:3). Sputum sample collected after a week also showed presence of similar structures and subsequently grew similar fungal colonies. Examination of the slide culture of the isolate revealed features suggestive of *Exophiala* spp. The isolate was sent to the National Culture collection of pathogenic Fungi, Postgraduate Institute of Medical Education and Research, Chandigarh for species identification and confirmation. The patient was immediately

put on voriconazole 200 mg twice daily for 10 days. Following treatment, she showed improvement in her cough, fever subsided and the follow up sputum culture after 3 months showed absence of growth of the fungus.

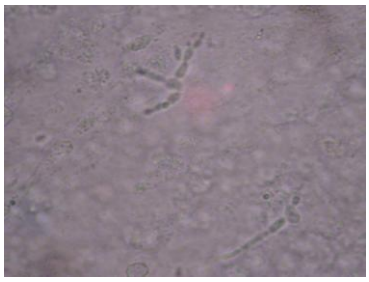

Fig 1. Direct mount of sputum showing hyphal elements

Fig 2. Dark brown colonies of *Exophiala mesophila* on Mycosel Agar

Fig 3. Microphotograph showing abundant yeast cells, with few hyphae and conidiogenous cells

Identification of the isolate

The isolate subcultured on Sabouraud's Dextrose agar (SDA) were initially mucoid white which later became black. The colonies became filamentous on further incubation with prominent dark brown diffusible pigment covering the entire media. Microscopic examination of the slide cultures of three week revealed abundant yeast cells, with few hyphae; conidiogenous cells which aroused directly from the hyphae were flask shaped with annelated zone. Ellipsoidal conidia of around 4 x 3 µm clustered around the conidiogenous cells or conidiophores (Fig-3). The fungus could grow in the presence of 1% cyclohexamide and up to 40^0C but was unable to grow at 42^0C. Molecular identification was done by sequencing the internal transcribed region (ITS) of rDNA including ITS1, 5.8S and ITS2. Amplification of this region was done by PCR using universal primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC). The amplified products were sequenced with Big Dye Terminator Cycle Sequencing Kit, Version 3.1(Applied Biosystems, CA, USA) for both the strands. The products were further purified and analyzed on ABI 3130 Genetic Analyzer (Applied Biosystems). Consensus sequences were obtained with the help of Bionumerics software (Version 6.5, Applied Maths, Ghent, Belgium). The sequence obtained was compared with that in the public database like GenBank and CBS data base. The sequence of our isolate gave 99% identity with *Exophiala mesophila*. By NCBI BLAST search our isolate showed 99% similarity with strain CBS-121507 (accession JF747120) whereas identification using CBS pairwise alignment showed 99% similarity with CBS 402.95 (accession CBS40295_3003_ITS) The strain is also deposited at NCCPF with accession NCCPF 106011. The Nucleotide sequence is deposited in GenBank with accession JN680269.

DISCUSSION

Phaeohyphomycosis is a cosmo-politan disease. Patients are usually adults and about half of them seem to be immunologically compromised or have associated underlying diseases such as

diabetes mellitus, tuberculosis, leprosy and cancer, tissue transplantation. The diagnosis of phaeohyphomycosis in predisposed patient population is facilitated by a high level of clinical suspicion. Its diagnosis must be confirmed by detecting the presence of phaeoid, pseudohyphal or hyphal elements in clinical material. [4] It is essential that the aetiology of cerebral and pulmonary phaeohyphomycosis in immuno-compromised patients must be establish on the basis of culture results. The isolate in our case was diagnosed to be *Exophiala mesophila* based on molecular characterization. Numerous members of Genus *Exophiala* are potential agents of human and animal mycosis and majority of these infections are cutaneous and superficial, but also fatal systemic infections are also reported. Several *Exophiala* species like *E. dermatitidis, E. oligosperma, E. phaeomuriformis, E. xenobiotica, E. lecanii-cornii* have been implicated in causing systemic diseases involving lung, pleural fluid, sputum etc. [2] However, *Exophiala mesophila* have been very scarcely reported to cause cutaneous and subcutaneous infection. [2] Extensive literature search to determine the role of *Exophiala mesophila* as a causative agent of pulmonary infection did not yield any results. As per our knowledge this appears to be the first case of *Exophiala mesophila* infection to be implicated in a patient with diabetes mellitus and pulmonary tuberculosis. This agent was isolated from a patient belonging to Assam state of Northeast of India, which lies in the tropical area. The climate is hot and humid and is conducive for growth of variety of fungi. This fact becomes all the more important if associated with the ever expanding group of patients who are immunocompromised.

Exophiala mesophila has been reported previously from silicone shower seals in a hospital ward in Hamburg, Germany [5] in treated dental unit waterlines [6] and in a retrospective study in 2007 as causative agent in cutaneous and subcutaneous infection. [2] *Exophiala* spp. are known to cause infection in people who are immunocompromised or otherwise. These are present in abundance in nature and require appropriate conditions to proliferate and cause systemic infection. Studies have reported *Exophiala* to be one of the three fungal genera to be disseminated efficiently by public drinking water [7] or proliferated in waterline that provide favourable condition for their growth. [6] Moreover, this group can grow at temperature of 32°C and upto pH of 9.5. [5] Such range of temperature along with possible polluted water supply and the suppressed immune status of the patient must have augmented the infection, survival and proliferation of the fungus in the patient in our case. It may be worthwhile to mention that majority of the population in the city are exposed to underground public and private drinking water supply.

Isolation of this rare *Exophiala spp*. as human pathogen involved in lung infection underscores the importance of early clinical suspicion in immunocompromised patient with chronic cough and requisition for fungal culture at the earliest. Isolation and characterization of the organism will not only help in appropriate treatment but also help throw light on the epidemiology of such rare opportunistic fungal pathogen.

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How to cite this article: Barua P, Rudramurthy SM, Devi U et. al. Isolation of *exophiala mesophila* from respiratory tract of an immunocompromised patient. Int J Health Sci Res. 2015; 5(3):449-453.

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