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## RESEARCH ARTICLE

# HYALOHYPHOMYCOSIS CAUSED BY PAECILOMYCES VARIOTII: CASE REPORTS AND IN VITRO SENSITIVITY

***Varsha Aglawe and Mubashir Azam Mir**

Department of Zoology and Biotechnology, Government Model Science (Autonomous) College Jabalpur, M.P. India 482001

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**ABSTRACT**

The hyalohyphomycosis are a group of diseases caused by filamentous fungi that are present in tissues as hyaline septate hyphae. *Paecilomyces* species cause various infections which are refered to as paecilomycosis. Here we report 2 cases of systemic mycosis in which *Paecilomyces variotii* was isolated from peripheral blood sample of 38 year old male and 42 year old female. Patient was diagnosed as infected hepatitis and post operative case of arm amputation and diabetic. The direct microscopic observation of the blood sample revealed many thin septate, branched mycelium. 5-Fluorocytosine was found to be the best effective drug.



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## INTRODUCTION

The term hyalohyphomycosisis used for those opportunistic mycotic infections which are caused by non-dematiaceous moulds that appear as hyaline hyphal elements in tissues (Ajello and Mcginnis 1984), and includes the terms such as fusariosis, penicilosis and paecilomycoses (McGinnis *et al.* 1985). *Paecilomycesvariotii* is a common ennvironmental mold found wide spread in composts, soils annd food products. *Paecilomyces* species are saprophytic fungi and are uncommonpathogens that can produce serious infections in immunocompromised patients and occasionally in immunocompetent hosts. *Paecilomycesvariotii* is a commonly occurring species in air and food. It is also associated with many types of human infections and is among the causative agents of opportunistic mycoses in immmunocompromised hosts. The apparent portals of entry for this organism are the respiratory tract, indwelling catheters and the skin (Walsh *etal.,* 1999, Groll *et al*. 2001, Pastor *et al*., 2006). *P. variotti* isan emergent pathogenic agent of pneumonia (Bruna *et al*., 2013).

***Corresponding author: Varsha Aglawe***
Department of Zoology and Biotechnology, Government Model Science (Autonomous) College Jabalpur, M.P. India 482001

An infection due to *P. variotii* in a diabetic patient who developed an upper lobe pulmonary infection has been reported (Byrd *et al*. 1992). Das *et al*. (2000) reported the first case of post transplantisolationn of *P.variotii* from the paediatric patient with cystic fibrosis. Lee *et al*. (2002) reported a case of sternotomy wound infection due to *P.variotii* in a previously bronchiectalic patient. Chamilos *et al*. (2005) reported a case of disseminated*P. variotii* infection in a neutropenic child with relapsed leukaemia who was on voriconazole prophylaxis. The *P. variotii* isolate was resistant to voriconazole in vitro.

### Case Report

### Case 1

A 38 year old male was admitted in the hospital, he had developed reduced appetite since last 8 days and fever with chills and shivering. Fever was sudden in onset intermittent high grade type followed by sweating.

He complained of vomiting 5-6 times in a day. He developed yellowish color of sclera to pass yellow colored urine. The patient was diagnosed as infected of hepatitis.

**Case 2**

A 42-year old female was admitted in the hospital with the complaints of weakness, tingling and numbness in lower extremities since past two years. Then she developed frequency of micturation polyphasia (increased appetite). Urine and blood examination showed increased sugar. She has been diagnosed as diabetic since last one year. She also complained loss of sensation in left middle and ring fingers and gradually ulcers developed on the fingers. There was blackish discoloration of fingers. Amputation below elbow was done 6 months back from the date of collection of sample. The patient was diagnosed as a post operative case of arm amputation and diabetes.

## MATRIALS AND METHODS

2ml. of blood from the patient was collected by means of vein puncture using sterilized needle and syringe. The blood was immediately transferred to a previously sterilized bottle containing 3 ml. of sabourauds dextrose broth and coated with heparin (anticoagulant). The bottle was agitated rapidly but gently to ensure proper mixing of the blood with anticoagulant. Sample was brought immediately to the laboratory. The bottle with suspension was incubated for 24 hrs at 28±1°C. In vitro susceptibility of the isolates to the antimycotics oxiconazole, amorolfine, 5 fluorocytosine, (5Fc), ketoconazole and amphotericin B (AmpB) was performed by threefold dilution method (Polak, 1983) on SDA medium. Stock solutions were prepared by dissolving the drugs in dimethylsulfoxide (DMSO) and then by further diluted in distilled water except 5-fluorocytosine which was dissolved directly in distilled water. The concentration of the drugstested ranged from 0.003 mcg $ml^{-1}$to 100 $mcgml^{-1}$ (1;3 dilution step) The test inoculums (predominantly conidia) prepared as described above contained $5.2x10^{4}$cfuμg $ml^{-1}$. Minimum inhibitory concentration (MIC) values of the drugs were recorded after 7 days of incubation at 28±1°C, MIC was defined as the lowest concentration of the drug allowing no growth of the fungus in duplicate determination.

## RESULTS

The blood sample showed thin walled, septate, branched mycelium in direct microscopic observation (Fig 1). On SDA at 28±1°C after 7 days of incubation, the colony was dark herbage green colour in periphery and olivaceous buff colour in the middle. The colony was rough in texture. The diameter of the colony at 28±1°C was 7×8 mm, 3×3 mm at 37 °C and no growth was observed at 40°C. Mycelium hyaline, septate, conidiophores arising directly from the mycelium bearing phialides in a loose verticillate group. Conidiophores 90 µm to 200µm × 2.5 µm to 3µm wide phialides upto7 in a whorl with cylindrical basal portion tapering little over half the length of a long slender type about 1µm wide. Conidia hyaline, single celled, smooth, globose to ellipsoidal 3µm to7.5µm × 1.5µm to 3µm in diameter (Fig 2). Of the antimycotics tasted in vitro 5-fluorocytosin was found to be the best effective drug, inhibiting growth of the fungus at 0.3 mcg $ml^{-1}$. Oxiconazole gave MIC of 10 mcg $ml^{-1}$and amorolfine and ketoconezole gave

MIC of 30 mcg $ml^{-1}$. Amphotericine B failed to inhibit the growth of *P. variotii* even at 100 mcg $ml^{-1}$.

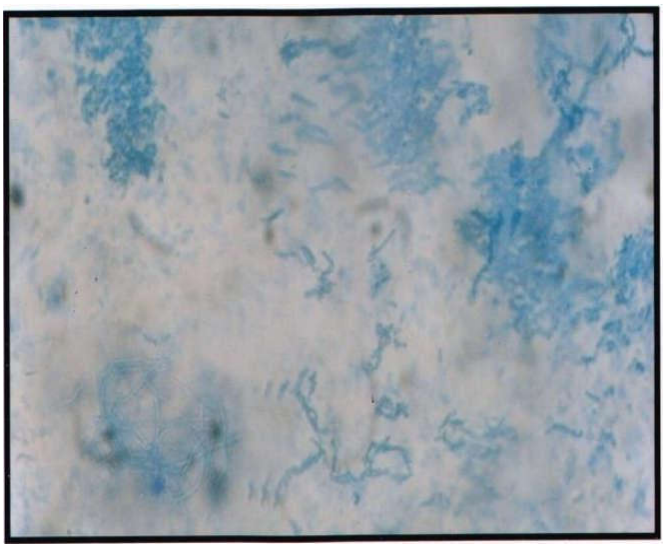

**Figure 1. DM of blood sample of P. variotii stained, 250 x**

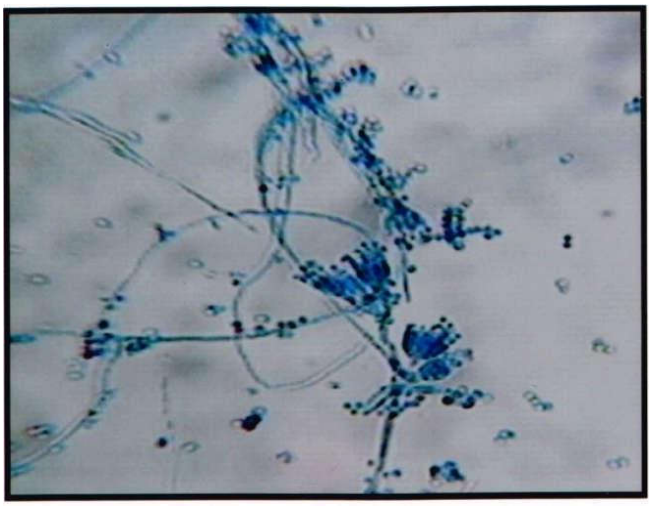

**Figure 2. Slide culture of P. variotii obtained from blood sample of Cotton blue stained, 400 x**

## CONCLUSION AND DISCUSSION

*Paecilomyces* species are found worldwide in soil anddecaying vegetation. Members of this genus are often encountered in the clinical microbiology as contaminants of cultured specimens. They are rare human pathogens. The first isolation of *Paecilomyces* species from humans was reported by Tu-ressen (Raper and Thom 1949) who isolated the fungus from human faeces. In 1950, a *Paecilomyces* species was isolated from human liver during a postmortem mycological study in USA (Haley and McCabe 1950). In 1971, a *Paecilomyces* species was isolated in 5 of 500 samples of sputafrom patients with chest diseases (Refai *et al*., 1971). In none of these reports was a pathogenic role attributed to the fungus. In 1963

*Paecilomyces variotii* was recognised as the causative agent of human disease for the first time when a case of fatal endo-carditis following mitral valve replacement was re-ported (Uys *et al*., 1963).

The present report shows that *P. variotii* is the etiologic agent of systemic mycosis in man. We isolated 2 cases of *P. variotii* from peripheral blood of the infected hepatitis and post operative diabetic patient. Earlier reports also documents disinfection in diabetic patient (Byrd *et al.,* 1992) and post lung transplant patient (Das *et al.,* 2000; Lee *et al.,* 2002). Castro *et al.,* isolated *P. lilacines* in a renal transplant patient. He concluded the anti fungal sensitivity varies widely among the species but the general trends are for *Paecilomyces variotii* isolates to be almost universally sensitive to amphoteric B and fluorocytosine. Unfortunately the different in vitro sensitivity testing methods for moulds are difficult to standardize (Kobayashi and Medoff, 1983). Moreover, there is in vitro and in vivo correlation of results on rare occasions only. However despite these drawbacks in vitro sensitivity testing of each clinical isolate is justified as it indicates a clear trend in susceptibility of the organism. This may help in adopting a definite line of therapy.

Data available on in vitro sensitivity testing of clinical isolates of *Paecilomyces*species indicate a definite trend in susceptibility to antifungal agents. *P. lilacinus* and *P. marquandii* are highly resistant to polyene antibiotics but are sensitive to imidazole while *P. variotii* almost universally susceptible to amphotericin B (Amp B) and fluorocytosine (5 Fc) (Shing *et al.,* 1996 and Aguilar *et al.,* 1998). The present isolate was found resistant to amphotericin B (Amp B) and susceptible to fluorocytosine (5 Fc). However, despite the relative resistance in vitro of *P. lilacinus* Amp B therapy has sometimes been reported to be successful. Castro *et al*. (1990) also found no in vitro and in vivo correlation ininfections by *P. lilacinus* and *P. variotii*. Unfortunately we cannot evaluate in vivo efficiency of antifungal drugs because the patient failed to return for therapy.

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