



Original Research Article

First Reported Human Case of Bilateral Subcutaneous Phaeohyphomycosis by a New Member of Dothideomycetes (*Pleosporales*) spp.

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ABSTRACT

A 45 years old female from Eastern India presented with painless multi-lobulated subcutaneous swellings on both forearms. She was controlled diabetic. From drained pus and biopsy materials dematiaceous fungal hyphae were demonstrated by KOH mount and PAS staining. Coffee brown colonies grew on SDA medium which showed brown branched hyphae with lateral anastomosis but no conidiogenesis. By molecular identification it was concluded as a new member of Dothideomycetes (*Pleosporales*) spp showing 86% sequence similarity with *Microsphaeropsis arundini*. By further morphological study bitunicate asci and asexual ascostromal development was noted. Thus it became first reported human case by such plant pathogen fungi. The probable reason for bilateral involvement might be due to her occupational trauma by carrying a type of elephant grass. The patient was managed by surgical excision and itraconazole therapy.

Keywords

Phaeohyphomycosis,
Dematiaceous,
Dothideomycetes,
Pleosporales,
Microsphaeropsis

Introduction

Melanized or dematiaceous fungi are ubiquitously present saprophytes and also enormously present on dead and living plants. The most common type of clinical presentation caused by dematiaceous fungi is phaeohyphomycosis of skin and subcutaneous tissue following minor, unrecognizable trauma by plant or vegetable material (Revankar and Sutton, 2010). In recent time many rare and new members of this group are emerging as important human pathogen. Apart from the common known

human pathogens like *Alternaria* spp., *Bipolaris* spp., *Exophiala* spp., *Cladosporium* spp., *Madurella* spp., *Sporothrix* spp., various black fungi belonging to Phylum Ascomycota, which are very new to medical mycologists are increasingly causing infections these days among immunocompromised as well as immunocompetent persons (Revankar and Sutton, 2010). It imposes difficulties in morphological identification and need for molecular methods in routine mycology

laboratory. Here we report the first case of bilateral subcutaneous phaeohyphomycosis by a new member of Dothideomycetes (Pleosporales) spp. in an immunocompetent host.

Case report

A 45 years old female from Howrah district, South Bengal, India presented with multilobulated subcutaneous swellings of about 5 cm sizes on dorsal as well as ventral aspects of both forearms (Fig. 1a). Lesions were chronically increasing for last eight months. There was no history of trauma, pain or fever. She was hypertensive and diabetic though her sugar level (fasting, postprandial, HBA1c level) was well controlled with medication at the time of presentation. All the relevant biochemical investigations were carried out which includes: Hb%, WBC total count, urea, creatinine, LFT, Lipid profile, RPR, Thyroid profile, serum cortisol level (Baseline and after dexamethasone challenge) as well as USG whole abdomen, Chest X-Ray, CT Scan of Brain. All reports were within normal limit. She was also non-reactive for HIV, HBsAg, anti-HCV tests.

We took pus and biopsy sample for investigation. The KOH mount of aspirated material revealed presence of brown septate branching hyphae, which were also PAS positive on staining (Fig 1b, 1c). Case was diagnosed as subcutaneous phaeohyphomycosis and itraconazole therapy was initiated empirically. Surgical excision of lesions was also undertaken.

Material was directly cultured onto Sabouraud's dextrose agar (SDA, HiMedia) and incubated at 25°C and 37°C. After 10 days of incubation both the tubes showed floccose to velutinous, dark mouse grey to coffee brown colonies (2–3.5 mm in

diameters). The reverse appeared leaden black to violaceous black (Fig. 2a,b). Lacto Phenol cotton blue preparation of the colonies showed thick and thin to dark golden brown as well as sub hyaline branching hyphae up to 5µm thick. Characteristic right angle branched hyphae with constriction at the site of origin & lateral anastomosis was observed (Fig. 2c). No conidia or asexual fruiting bodies were evident after prolonged incubation and subculture on cornmeal agar (CMA, HiMedia). Similar fungi isolated from lesions on both the hands and also present in histopathological examination (Fig. 1d). Based on the morphological characters we provisionally diagnosed it as *Rhizoctonia* spp. though we failed to find sclerotia. Then the isolate was subcultured on SDA and sent to National Fungal Culture Collection, India (NFCCI) at Pune for molecular identification and also deposition of the strain in the culture collection with unique accession number. After sequencing the isolate (NCBI accession JQ759930.1/ isolate FL0071/ Dothideomycetes sp. Genotype 223) showed 86% sequence similarity with *Microsphaeropsis arundinis* and diagnosed as a new member of Dothideomycetes (Pleosporales) family (Fig. 3). Then we started further morphological studies after prolonged incubation and appreciated few structures like bitunicate asci, multi-cellular fruiting body arising from hyphae (Fig. 4) but failed to find any pycnidium.

Following surgical excision itraconazole therapy continued for 3 months, patient responded and no recurrence reported within one year of follow up period.

Dothideomycetes is a diverse class of ascomycetes fungi, characterized with bitunicate (thick, double layered) asci without any apical pore and ascostromatic or ascolocular development of pseudothecia

(perithecia initiated from non-sexual vegetative hyphal cells (Schoch *et al.*, 2009). Predominantly plant pathogen this class of fungi is divided into several subclasses and orders though poorly understood till date. Amongst the seven orders, *Pleosporales* comprises the commonest causative agents of phaeohyphomycosis. Besides the well-known melanized anamorphic hyphomycetes e.g. *Alternaria*, *Curvularia* few unusual coelomycetes e.g. *Microsphaeropsis*, *Phoma*, *Pleurophoma* are sporadically reported as human pathogen (Guarro *et al.*, 1999; Shah *et al.*, 2001; Padhye *et al.*, 2004; Stella *et al.*, 2004; Galipothu *et al.*, 2015).

A heterogenous group of Ascomycotic fungi are called as 'Mitosporic Ascomycota' due to absence of any sexual stage. *Microsphaeropsis* is a member of this group and also comes under coelomycetes group as conidia bourn inside closed sac which forms only asexual fruiting body, called Pycnidia with small aseptate conidia (Sutton, 1980). Pycnidium is an asexual version of perithecium, as the first one produces conidia and the other produces sexual ascospores.

Different species of *Microsphaeropsis* are identified on the basis of structural difference of pycnidia and conidia. But production of pycnidium is very difficult and slow, needs various special sporulating culture media like carnation leaf agar and long term incubation.

In our routine laboratory with only two simple fungal media in hand, it was not possible to sporulate the isolate. Previous reports expressed such difficulties and took help of reference laboratory of morpho-identification of such isolates (Stella *et al.*, 2004). Therefore molecular techniques are the new tool to solve the mystery of plant

pathogen fungus which are affecting human newly.

Kingdom: Fungi

Phylum: Ascomycota

Class: Dothideomycetes

Order: Pleosporales

Family/Clade: Didymellaceae

Genera: *Microsphaeropsis*

Species:

Microsphaeropsis arundinis

Microsphaeropsis olivaces

Microsphaeropsis callista

Microsphaeropsis conielloides

Phylogenetic tree of *Microsphaeropsis* according to multi-locus DNA sequencing (Zhang *et al.*, 2009).

Our case was apparently healthy patient without any systemic disease presented with bilateral lesions and there was no organ dissemination found after radiological investigations. Meticulous history taking uncover her habit of carrying bundles of 'hogla leaves' on her forearm leading to minor trauma. 'Hogla' plants (*Typha elephantina*) or elephant grass, a gigantic marsh plant luxuriously grown in canals, roadside shallow water of southern Bengal is used for thaching. Literature study revealed *Microsphaeropsis arundinis* was first isolated from *Arundo donax*, similar type of grass growing in wetlands (Ahmad, 1971) and thereafter many marine plants (Wanderley Costa *et al.*, 2012). Further research may explore relation of this fungus with hogla plant. In present case bilateral traumatic implantation might be possible reason for bilateral disease. In subcutaneous mycoses among immune-competent bilateral incidence is exception rather than rule (Maiti *et al.*, 2000).

Fig.1(a) Multi-lobulated subcutaneous lesions before treatment. (b) KOH mount of pus showed brown septate branching hyphae (400x). (c) Pas positive branching fungal hyphae (1000x). (d) Fungal elements present in histopathological slide (1000x).

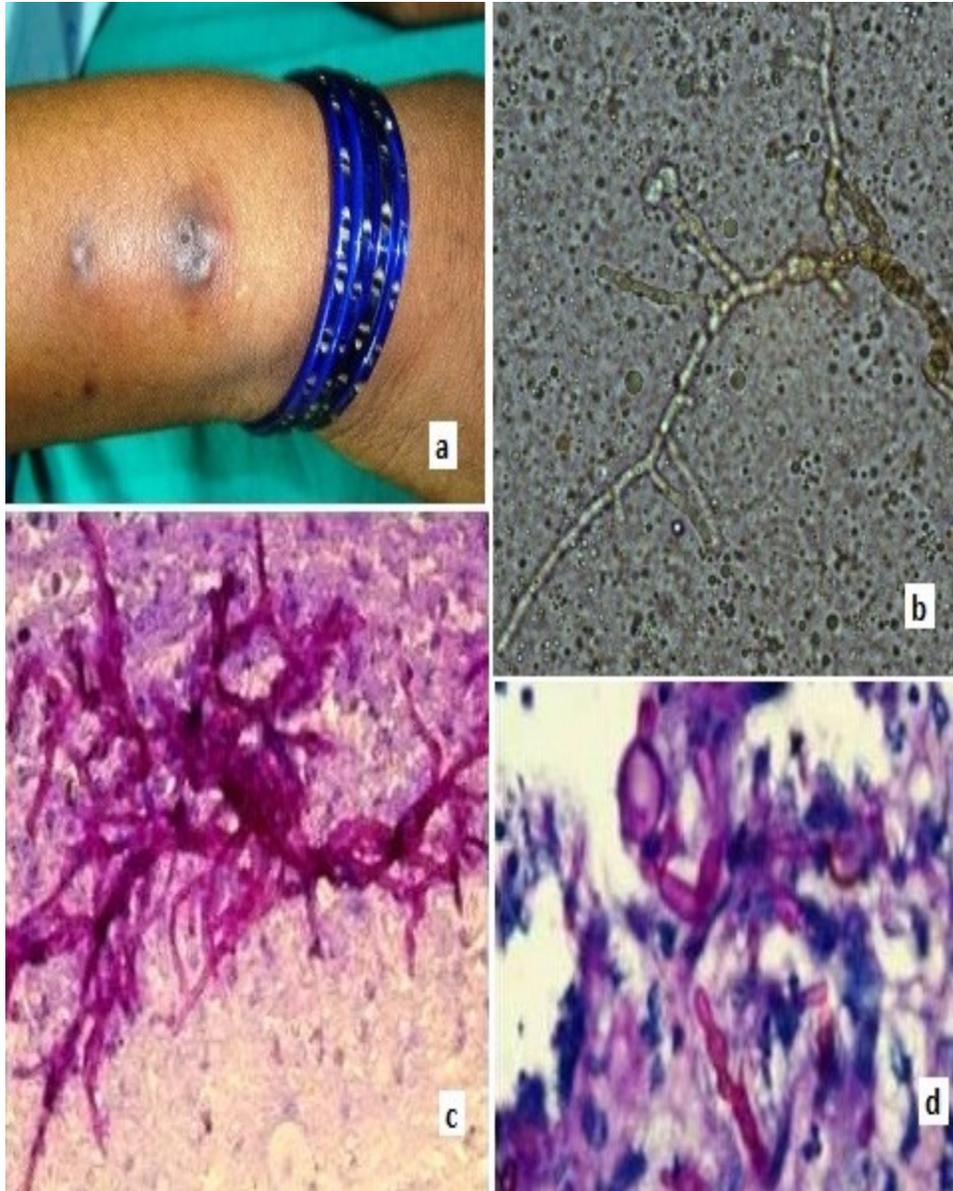


Fig.2 (a) and (b) Obverse and reverse side of dematiaceous fungal colony on Sabouraud's dextrose agar. (c) Lacto Phenol cotton blue preparation of the colonies showed brown right angle branching hyphae with lateral anastomosis (400x)

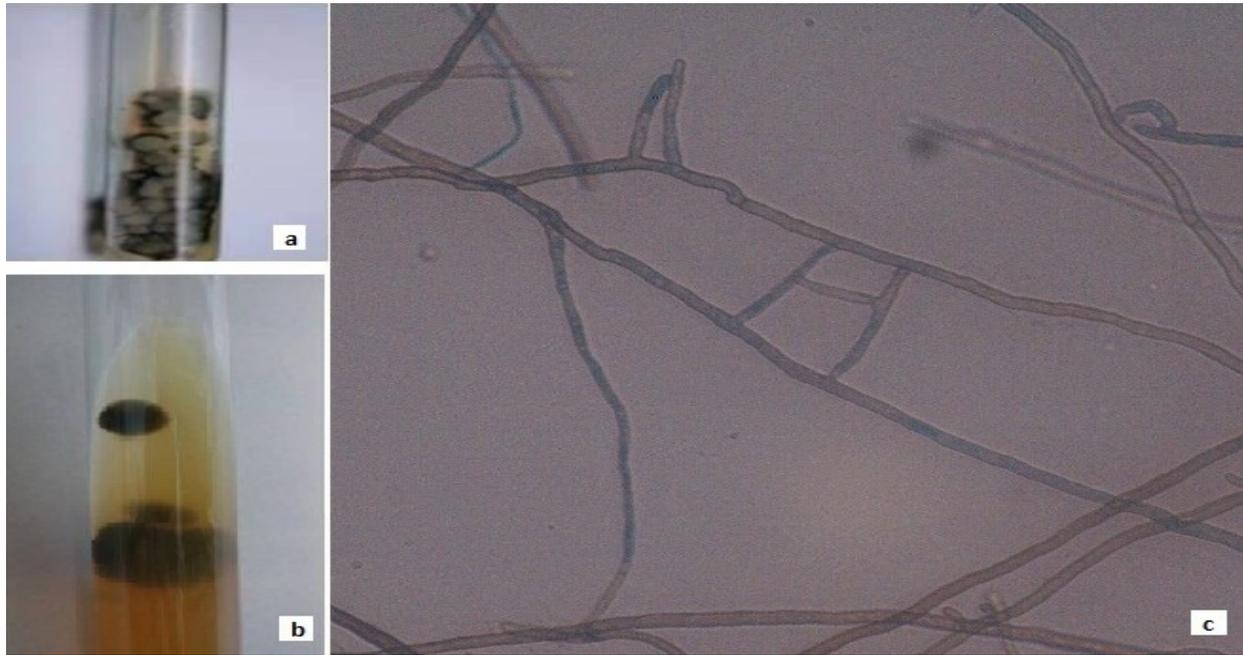
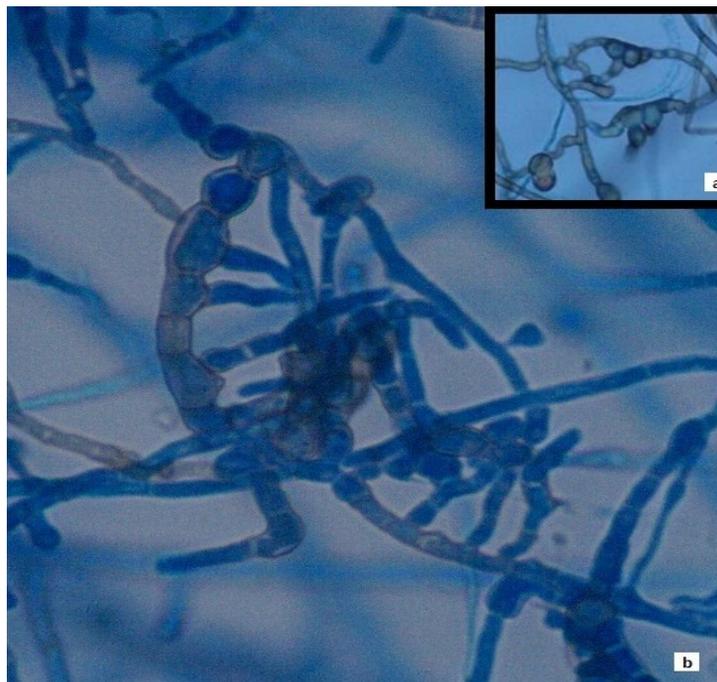


Fig.3 Sequence analysis of ~500bp rDNA fragment showed 86% sequence similarity with *Microspheeropsis arundinis*. Possibly a new member of Dothideomycetes (Pleosporales) spp

SEQUENCE ANALYSIS (507 bases) with NCBI sequence Accession JQ759930.1
 NCBI Accession JQ759930.1/ isolate FL0071/ Dothideomycetes sp. genotype 223
 Length=1112
 Score = 381 bits (206), Expect = 3e-102
 Identities = 326/380 (86%), Gaps = 24/380 (6%)
 Strand=Plus/Plus

Query	130	TTACAAC	TTTCAACA	ATGGATC	TCTTGGCT	CTGGCCAT	CGATGAAGA	AACGCAGC	GAAATGC	189		
Sbjct	201	TTACAAC	TTTCAACA	ATGGATC	TCTTGGCT	CTGGCCAT	CGATGAAGA	AACGCAGC	GAAATGC	260		
Query	190	GATAAC	GTAGTGT	GAAATGC	CAGATTCC	AGTGAATC	ATCGAATC	TTTGAAC	GCACAT	TGCG	249	
Sbjct	261	GATAA-	GTAGTGT	GAAATGC	AGAATTCA	GTGAATC	ATCGAATC	TTTGAAC	GCACAT	TGCG	319	
Query	250	CCCCTT	GGTATT	TCCAAAG	GGCATGC	CCTGTT	CGAGCGT	CATGGAC	CACACC	CTCAAG	CTCTG	309
Sbjct	320	CCCCTT	GGTATT	TCCATGG	GGCATGC	CCTGTT	CGAGCGT	CATCTAC	AC-CC-	TCAAGC	CTCTG	377
Query	310	CTTGGT	GTTGGG	CGGCTG	TTCCCG	CATGCG	TGAGGGG	ACTCGC	CTCAA	AGGCAT	TGGC	369
Sbjct	378	CTTGGT	GTTGGG	CGTCTGT	-CCC-	GCCTC-	CGCGCG	TGGACT	CGCCCC	AAATGC	ATTGGC	434
Query	370	AGCTGG	GCTGT	CTGCC-	CCTCTC	TGCGCC	TAGCTTT	TGCGCG	-CAAC	GGGGG	TGGGCC	427
Sbjct	435	AGC-GGT	TCT-T-	TGCTCCT	CCTC-	GCGCAG	CA-TT-	GCGCTT	CAGAG	GGGCG	TGGGCC	488
Query	428	GGCAGC	CGTAGC	AAACCAG	CAAGCAT	GAACCAG	CTTTT	TGACCT	CGAATC	AGGTAG	GAGTA	487
Sbjct	489	G-CGTCC	--A-	CGA--	AGCAA-	CATTA-	CC-G-	TCTT	TGACCT	CGGATC	AGGTAG	538
Query	488	CCCCTG	AACTTA	AGCATAT							507	
Sbjct	539	CCCCTG	AACTTA	AGCATAT							558	

Fig.4(a) Bitunicate asci, (b) multi-cellular fruiting body arising from hyphae were observed under microscope (400x)



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