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Website: www.jacmjournal.org

DOI: 10.4103/jacm.jacm_3_17

Lichtheimia ramosa isolated from a young patient from an infected wound after a road traffic accident

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Abstract:

A 38 year-old male, a known case of diabetes mellitus on treatment, sustained an injury to his left foot in a Road Traffic Accident (RTA) and developed a polymicrobial wound infection. The causative organisms were found to be *Pseudomonas aeruginosa* along with a Zygomycete which could not be speciated by conventional microscopy but was identified as *Lichtheimia ramosa* by Polymerase Chain Reaction (PCR) and Internal Transcribed Spacer (ITS)sequencing. The infection was treated successfully by debridement and daily dressing with antiseptic solution. The patient recovered due to early diagnosis and did not require systemic antifungals.

Keywords:

Diabetes mellitus, fungal infection, road traffic accident

Introduction

Mucormycosis is a potentially fatal fungal infection that occurs in immunocompromised patients, diabetic patients with ketoacidosis and immunocompetent patients after trauma and exposure to contaminated soil.[1] These filamentous fungi belong to the order Mucorales. About twenty different species have been shown to be pathogenic for humans.[2] Other genera causing mucormycosis are *Rhizopus* spp., *Mucor* spp., and *Cunninghamella* spp., while *Apophysomyces* elegans and *Lichtheimia* spp. are less commonly reported.[1] However, information on exact incidence of disease is limited because of several factors. The identification up to the species level needs expertise usually available only at reference laboratories.

In our case study, we describe a *Lichtheimia ramosa* mycosis in a patient who had severe traumatic injury to his left foot because of a road traffic accident (RTA). The etiological agent was identified morphologically and molecularly. Apart from *Lichtheimia corymbifera*, there is another species within the genus, *L. ramosa* which is responsible for mucormycosis in humans. These two species can be differentiated based on their molecular, biological and morphological characteristics.

Case Report

A 38-year-old male hailing from Sankarapuram Taluk, Viluppuram District, Tamil Nadu State, India, had been admitted to our hospital with the complaints of ulcer over the left foot since one month. The patient gave a history of RTA a month back in his hometown where he sustained a laceration injury to the left foot which was sutured at the time without proper cleaning in a nearby local hospital. The patient was also a known case of diabetes mellitus since 20 years on regular oral anti-glycaemic medications. A general examination found the patient to be

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How to cite this article: Neelaveni V, Tupaki-Sreepurna A, Thanneru V, Kindo AJ. *Lichtheimia ramosa* isolated from a young patient from an infected wound after a road traffic accident. J Acad Clin Microbiol 2017;19:59-61.

conscious, oriented, afebrile and normotensive with stable vitals. Systemic examination was found to be normal.

Local examination of the left foot showed a non-healing ulcer present in the plantar aspect of the left foot 7 cm × 3 cm × 2 cm, purulent discharge present, slough present [Figure 1]. A pus swab/aspirate was sent to the microbiology laboratory. The wound was subjected to daily thorough cleansing with antiseptic solution (Povidone-iodine 10%; Betadine) and surgical debridement every two to three days and left to heal by secondary intention.

Laboratory investigations

Gram stain of pus smear showed moderate number of pus cells and a few Gram-negative bacilli. The KOH mount of the specimen showed broad aseptate fungal elements. Pus culture grew *Pseudomonas aeruginosa* along with a mould. Specimen was collected under aseptic precautions and cultured on Sabouraud dextrose agar (SDA) and incubated at 37°C for four to five days which yielded a filamentous fungus with rapid growth. The growth on Potato dextrose agar was within 72 h; colonies were woolly, initially white and then changed to grey [Figure 2a].

On SDA, fungus grew rapidly as cottony colonies after incubation at 37°C for 48 h. Microscopic examination showed branching aseptate broad hyphae. The sporangiophores were highly branched and terminated in sporangia or the columellar remnants. Most branches were arranged in umbel shape, usually at the terminals [Figure 2b]. In young colonies, circinate sporangiophores were seen under the microscope using a ×10 objective. No rhizoids were observed. The sporangia were hyaline, multispored and pyriform in shape. The columella had prominent flask-shaped apophysis which was dome shape [Figure 2c]. Hyaline sporangiospores found inside sporangia were smooth and ellipsoidal in shape.

Figure 1: Local examination of the left foot showing a non-healing ulcer present on the plantar aspect of the left foot 7 cm × 3 cm × 2 cm, purulent discharge present, slough present

Pleomorphic giant cells with finger-like projections were observed, submerged in the agar, which was a characteristic of this genus [Figure 2d]. Giant cells are seen in all species of *Lichtheima*. Giant cells are strongly swollen, branched or unbranched, often droplet-filled hyphae with thick, refractive walls. They are frequently septate and branched. Some giant cells possess projections. Based on the phenotypic characteristics, the isolate was reported as *Lichtheimia* species.

Molecular speciation

DNA extraction was done directly from the culture plate by an in-house column-based method. Polymerase chain reaction amplification of a partial region of the internal transcribed spacer (ITS) nuclear ribosomal RNA gene (ITS1, 5.8S rDNA, ITS2) was performed using the pan-fungal primers ITS1 (5′ TCC GTA GGT GAA CCT TGC GG 3′) and ITS4 (5′TCC TCC GCT TAT TGA TAT GC 3′). Gene sequencing was done at SciGenom Labs Pvt. Ltd., Cochin, Kerala, India. The ITS sequence identified the isolate to be *L. ramosa* and has been deposited in the National Center for Biotechnology Information GenBank database with accession number KX236396.

>LICH_ITS1.FORWARD_27720-1_8381, Trimmed_Sequence_(685 bp)CTGCCT TGTGCTGTAAATCTGGTTGTTGGCATAAAA ACCCTCCTTTTAGGAAACTTGTGCCA CTACTAAAATCTAGGCTGCTTGAAAAAAAAC ATATGGACCCTTCTTTCAGGAGACCT ATGTCTCGAGTCAAACCAAGCAAG GCAAGCCTTTGGGGCTCTAGTACTAACT ATCCCCAAAGGTGTTTATTCTTCTCGTGTAAACC

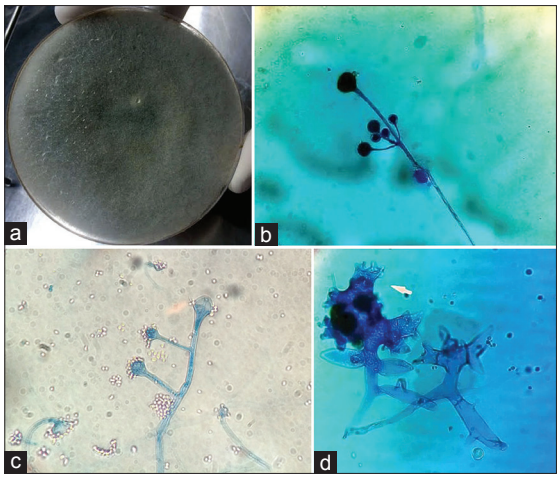

Figure 2: (a) Culture on Potato dextrose agar was fast growing, cottony to fluffy, white to yellow, becoming dark grey. (b) Microscopy of growth from Sabouraud dextrose agar medium showing branched sporangiophores arranged in umbel shape, usually at the terminals (×100). (c) Microscopy of growth from Sabouraud dextrose agar medium showing columella with prominent dome-shaped apophysis and hyaline spores (×100). (d) Microscopy of growth from Sabouraud dextrose agar medium showing abundant pleomorphic giant cells with finger-like projections submerged in agar (×100)

ATGATGTACGAAAAAGTTAGTTGTTA
ACTTAAAAACAACTCTTGGCAATGGAT
CTCTTGGTTCTCGCATCGATGAA
TAGCGTACCAAAGTGCGATAAT
TATTGCGAC TTGCATTCATAGCGAATCATC
CAGTTCTCTAACGCATCTTGCGCCT
AGTAATCAATCTACTAGGCAC
AGTTGTTTCATTATCTGCAACTACCA
ATCAGTTCAACTTGGTTCTTTGAACCTAA
CCGAGCTGGAAATGGGCTTGTGTTGATG
GCATTCATTTGCTGTCATGGCCTTA
AATACATTTAGTCCTAGGCAA
TTGGCTTTATTCATTTGCCGGATGTAGAC
TCTAGAGTGCCTGAGGAGCAACGACTTG
GTTAGTGAGTTCATAATTCCAAGTCAATCAGTC
TCTTCTTGAACTAGGTCTTAATCTTTACGG
ACTAGCGAGAGGATCTAACTTGGGTCTTC
TCTTAACAAACTCACATCTAGATCTGAA.

Discussion

The genus *Lichtheimia* was recently revised on the basis of phylogenetical, physiological and morphological characteristics.[3] The thermotolerant species of *L. corymbifera* (formerly *Absidia corymbifera*), *Lichtheimia blakesleeana* and *Lichtheimia hyalospora* were placed in the new family Mycocladiaceae and the genus *Mycocladus*. More recently in the nomenclature correction, a family Lichtheimiaceae was created instead of Mycocladiaceae.[4] The genus *Lichtheimia* belongs to the order Mucorales and includes saprotrophs isolated from soil, decaying plant material or dung.[5,6] Three out of five currently accepted species, namely, *L. corymbifera*, *Lichtheimia ornata* and *Lichtheimia ramosa* , are known to cause human infections (mucormycoses) predominantly in patients with impaired immune systems.[7] The majority of cases caused by *Lichtheimia* species relate to patients that are severely debilitated due to malignancies, poorly controlled diabetes or solid organ transplantation. Cutaneous, pulmonary, rhinocerebral, renal and disseminated infections as well as otomycosis have been described,[8] and hence, the spectrum of infections due to *Lichtheimia* spp. is similar to that of other members of the family Mucorales. Soil serves as habitat and spore reservoir for *Lichtheimia* species. Several cases are known where traumatic injuries contaminated with soil resulted in *Lichtheimia* infections in immunocompetent patient.[9] *L. corymbifera* was until recently, the only species in this genus known to be disease causing, and it accounts for approximately 5% of mucormycosis today.[10] However, the true prevalence is unclear.[11] The use of molecular identification[12] is important for an accurate assessment of the epidemiology.

In the present case, traumatic implantation of *L. ramosa* caused local mucormycosis in an immunocompetent patient. Although both medical and surgical treatment are usually required, in our case, the patient was diagnosed in an early stage and was successfully managed with surgical debridement combined with irrigation with amphotericin B, without the need for systemic antimycotics. Bibashi *et al*.[13] described a mycosis due to *L. corymbifera* in a young male patient with multiple traumatic fractures that were also healed by surgical debridement and revascularization only.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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