

**Case Report****A Rare Case of *Curvularia Hawaiiensis* in the Ear Following Trauma**

Authors

Dr Santhi Gunasekaran¹, Dr Ananya Tupaki-Sreepurna², Mr Vijayakishore Thanneru³, Dr Uma Sekar⁴, Dr Mariappan Shanthy⁵, Dr Anupma Jyoti Kindo⁶¹MD Microbiology Post Graduate, Department of Microbiology²MD Microbiology, PhD Scholar, Department of Microbiology³MSc Medical Microbiology and Applied Molecular Biology, Department of Microbiology⁴MD Microbiology, DCP Professor & Director Clinical Laboratory Services, Department of Microbiology⁵MD PhD Microbiology, Associate Professor, Department of Microbiology⁶MD Microbiology, Professor & Head, Department of Microbiology, Sri Ramachandra Medical College & Research Institute, Porur, Chennai-600116

Corresponding Author

Dr Santhi Gunasekaran

No:1/23, Pillayar Koil Street, Ayyappanthangal, Chennai-600056

Email: radhikaguna1989@yahoo.co.in, +919789907919**Abstract**

Curvularia belongs to the group of phaeoid or black fungi that can cause opportunistic infections in humans and other animals. *Curvularia hawaiiensis* or *Cochliobolus hawaiiensis* (previously *Bipolaris hawaiiensis*) is mainly a plant pathogen which is commonly isolated from soil and vegetative matter. We report here a rare case of Chronic Suppurative Otitis Media (CSOM) of the ear caused by *Curvularia hawaiiensis* in a young male after a Road Traffic Accident (RTA). The isolate was identified by a combined approach of morphological and molecular methods using Internal Transcribed Spacer (ITS) rDNA sequencing. The species previously belonged to the genus *Bipolaris* and was known as *Bipolaris hawaiiensis* but has recently been phylogenetically reclassified to fit into genus *Curvularia*. The patient failed to return for follow-up but was traced and called to return when confirmed to be symptomatic. He was later treated with topical itraconazole.

Case Report

A 38-year-old previously healthy male who worked as a construction site labourer was brought to the casualty ward of our hospital with a history of loss of consciousness and right ear bleed following a road traffic accident. In the casualty, the patient was conscious and oriented, with a Glasgow Coma Score (GCS) within normal

limits. Preliminary investigations including a Computerized Tomography (CT) scan were also normal. He was admitted as an inpatient for further monitoring and evaluation. Routine laboratory investigations were done. An ear swab was also sent for bacterial culture and sensitivity. There were no microorganisms seen in the Gram Stain and the culture plates had no growth in 48

hours. Patient was doing well and was discharged on 12/2/17 and was asked to come after a month for follow up. On 15/2/17, we observed both Blood agar and MacConkey agar culture plates growing a greyish black mould which seemed to a *Bipolaris* species on LactoPhenol Cotton Blue (LPCB) wet mount and was reported as such. Subsequently, the growth was subcultured on Sabouraud Dextrose Agar (SDA) (HiMedia Laboratories, Mumbai, India) and plates were incubated at both 25 and 37⁰C temperature. The plates grew a mold, white during the earlier stages, turning into grey and then black on further incubation. The colonies were spreading, suede-like to downy, dark grey to black, with a black reverse ^[1,2] and more luxuriant at 25 than 37⁰C

temperature (Figure 1). A slide culture was put up using Oatmeal agar (HiMedia Laboratories, Mumbai, India) and was observed after ten days growth. Microscopy revealed conidiophores that were erect to apically flexuous, septate, unbranched with flat conidial scars on the edges, producing conidia in sympodial succession. Conidia were ellipsoidal, sometimes lunate, rounded at one end and tapering slightly towards the base, pale to dark brown with 3-5 distosepta, with conidial wall smooth to verrucose. The hilum was not found to be protuberant ^[1,2] (Figure 2). The isolate was classified as genus *Bipolaris* but could not be resolved decisively up to the species level.

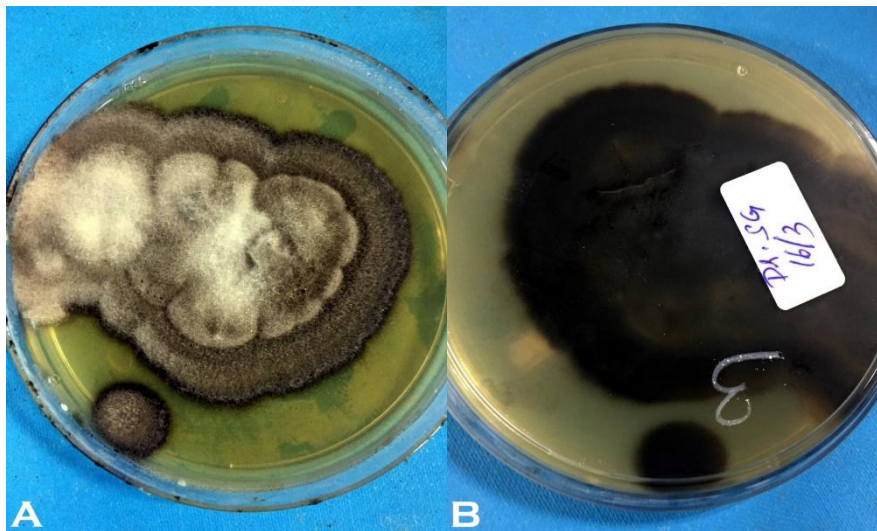


Figure 1: Growth of *Curvularia hawaiiensis* on Sabouraud Dextrose Agar: spreading, suede-like to downy, dark grey to black colonies (A) with a black reverse (B)



Figure 2: Microscopic features of *Curvularia hawaiiensis*, septate unbranched geniculate conidiophore with brown ellipsoidal to lunate conidia with 3-5 distosepta

The culture report was informed to the ENT consultant. Although the patient had been discharged by then, he was expected for a follow-up visit in the next couple of weeks. We proceeded with the molecular speciation of the isolate since results of morphological study were inconclusive.

Molecular identification involved extraction of fungal genomic DNA, amplification of the ITS target region, followed by nucleic acid sequencing and analysis. Extraction of fungal genomic DNA (Deoxyribonucleic acid) was done by a column based in-house method standardized in our lab^[3, 4]. Briefly, a small piece of fungal culture (1x1cm²) was scraped off from a fresh culture on Soyabean Casein Digest Medium (SCDM) agar (HiMedia Laboratories, Mumbai, India) or Sabouraud Dextrose Agar (SDA) plate and finely ground with a mortar and pestle by adding 0.5ml of TESS (Tris-EDTA-Sodium chloride-SDS) buffer. TESS buffer was prepared in the lab with Tris base, ethylenediaminetetraacetic acid, sodium chloride and sodium dodecyl sulphate and used as the lysis buffer. The grinding step was incorporated to break down the fungal mycelium for the action of lysis buffer. After grinding, the mould was collected into a 2ml microcentrifuge tube and subjected to treatment with Proteinase K at 56⁰C for half an hour. Further steps included treatment with absolute alcohol, wash buffer 1 (guanidine hydrochloride), wash buffer 2 (Tris-HCl) and finally elution buffer (nuclease free water) to obtain 50µl of pure genomic fungal DNA. For DNA amplification, the panfungal marker Internal Transcribed Spacer (ITS) region was the target region selected and the primers used were ITS1 5'-TCCGTAGGTGAACCTGCGG-3' and ITS 4 5'-TCCTCCGCTTATTGATATGC-3' which amplified partial region of the 18s rRNA, the ITS1 region, 5.8s rRNA, ITS2 and partial region of the 28s rRNA region. PCR reactions (volume 50 µl) were performed in a thermocycler using 1 cycle at 95⁰C for 3 minutes, followed by 35 cycles with a denaturation step at 95⁰C for 30 seconds, an

annealing step at 55⁰C for 30 seconds, an extension step at 72⁰C for 30 seconds and a final extension step at 72⁰C for 10 minutes. A negative control was included in the reaction. Amplicon detection was performed by electrophoresis of an aliquot of 10µl of each amplicon in a 1.5% agarose gel with ethidium bromide 0.02% in 1x Tris-acetate-EDTA (TAE) buffer. The DNA bands were then visualized under UV illumination. A 1kb molecular weight ladder was used for reference. The PCR amplicon was sequenced at AgriGenome Labs, Cochin, Kerala, India using the Big dye terminator V.3.1 cycle sequencing kit and software Sequencher. The nucleic acid sequence obtained was used for nucleotide - nucleotide search and comparative analysis using the Basic Local Alignment Search Tool (BLAST) algorithm at the National Centre for Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov/BLAST/>) and the ISHAM-ITS online database (<http://its.mycologylab.org/>). Hits more than 99% were considered. Matches with reference strains from culture collection were chosen. The ITS sequence obtained for the isolate provided multiple 100% matches with *Curvularia hawaiiensis* strains and was deposited at the NCBI GenBank database with the accession number MF034504.

Because the patient failed to appear at a follow-up appointment during the expected time, he was contacted by phone and was requested to do so. On being asked if he had any ear complaints during his visit, he described not being able to hear as well with his right ear when compared to the left. It was understood that the patient had been hard of hearing for the last two months and had assumed it to be unrelated to his recent RTA. He was subsequently started on topical itraconazole therapy and is currently undergoing the same^[5].

Discussion

Curvularia belongs to the dark pigmented or melanized group called phaeoid or dematiaceous

fungi that include genus *Bipolaris*, *Alternaria*, *Exophiala*, *Exserohilum*, *Scedosporium*, *Cladophialophora*, *Fonsecaea* and many more. Melanin, an extremely stable resistant molecule, is involved in the pathogenicity^[6]. Infections caused by these black fungi are termed phaeohyphomycosis and comprise mainly of ulcerative skin lesions of traumatic origin, black grain eumycotic mycetoma, fungal keratitis, paranasal sinusitis and rarely systemic (respiratory and cerebral) or disseminated disease^[6,7].

With regard to *Curvularia*, there have been sporadic reports of deep infections such as endophthalmitis, fatal recurrent *Curvularia* brain abscess in a child and *Curvularia* endocarditis following cardiac surgery^[6,7,8] where none of the patients suffered from known immunologic disorders or underlying debilitating diseases. A fatal case of cerebral *Curvularia* infection was also reported in a young african american male with no known history of immunocompromise or prior respiratory tract or sinus infection in 2004^[9]. *Curvularia* and other fungi were seen to colonise the hollow insides of wind instruments like the saxophone, clarinet and flute, leading to hypersensitivity pneumonitis or 'saxophone lung' in the players, an allergic reaction with chronic coughing and wheezing^[10,11]. Cutaneous *Curvularia* lesions have also been reported and the fungus is observed to grow well on keratotic material^[12]. The genus *Curvularia* contains about 80 different species, which are mostly present in soil and plant material. Previously, *Curvularia lunata* was believed to be the most frequently reported human pathogenic species. However, other species such as *C. americana*, *C. brachyspora*, *C. chlamydospora*, *C. clavata*, *C. hominis*, *C. inaequalis*, *C. muehlenbeckiae*, *C. pallescens*, *C. pseudolunata*, *C. senegalensis* and *C. verruculosa* have now also been reported from clinical cases^[6,13,14,15]. Recent studies have shown that morphological identification of *Curvularia* species does not correlate with molecular speciation^[13,14,15]. The different species within *Curvularia* resemble each other too closely to be

able to be identified morphologically. Molecular techniques like DNA sequencing serve as a useful tool in these cases. Recently, the phylogenetic analysis of the genera *Bipolaris* and *Curvularia* has resulted in a re-alignment of several species. In particular, clinical isolates previously identified as *Bipolaris* species, notably *B. australiensis*, *B. hawaiiensis* and *B. spicifera* have now been transferred to *Curvularia*^[15].

The true prevalence of the different species of *Curvularia* in human infections is unknown since only a few studies involving this genus have been published and the isolates were usually identified only by morphological criteria till recent years. Considering the similarity among the species of *Bipolaris* and the fact that the separation of species is based on subtle characters, some published identifications are doubtful or remain unresolved. In the present study, we identified *Curvularia hawaiiensis* not only with the help of cultural characteristics but also with DNA sequencing and this rare pathogen was found to be the cause of CSOM of the ear, a previously undescribed presentation of infection with this genus.

Conclusion

Phaeoid fungi cause phaeohyphomycosis and are opportunistic human pathogens of emerging importance. Accurate identification of the etiological agent requires a combined approach using morphological and molecular methods. Here we report a rare presentation of *Curvularia* infection in the form of CSOM, where the pathogen was isolated from the ear of a patient post-trauma. The patient was begun treatment with itraconazole.

Conflicts of Interest: Nil

Acknowledgements: Nil

Contributions of Authors

All six authors contributed equally to this work.

References

1. Manamgoda DS, Cai L, Bahkali AH, Chukeatirote E, Hyde KD. *Cochliobolus*: an overview and current status of species. *Fungal Divers*. 2011 Dec 1;51(1):3-42.
2. de Hoog GS, Guarro J. Atlas of clinical fungi. Centraalbureau voor Schimmelcultures; 1995.
3. 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 Jan 1;19(1):59.
4. Chadiesh N, Jishnu BT, Tupaki-Sreepurna A, Ramanan R, Thanneru V, Kindo AJ. *Aspergillus quadrilineatus* infection in an elderly debilitated patient. *J Acad Clin Microbiol*. 2017 Jan 1;19(1):62.
5. Safdar A. *Curvularia*—favorable response to oral itraconazole therapy in two patients with locally invasive phaeohyphomycosis. *Clin Microbiol Infect*. 2003 Dec 1;9(12):1219-23.
6. Revankar SG, Sutton DA. Melanized fungi in human disease. *Clin. Microbiol. Rev*. 2010 Oct 1;23(4):884-928.
7. Revankar SG, Patterson JE, Sutton DA, Pullen R, Rinaldi MG. Disseminated phaeohyphomycosis: review of an emerging mycosis. *Clin. Infect. Dis*. 2002 Feb 15;467-76.
8. Rinaldi MG, Phillips P, Schwartz JG, Winn RE, Holt GR, Shagets FW, Elrod J, Nishioka G, Aufdemorte TB. Human *Curvularia* infections: report of five cases and review of the literature. *Diagn Microbiol Infect Dis*. 1987 Jan 1;6(1):27-39.
9. Carter E, Boudreaux C. Fatal cerebral phaeohyphomycosis due to *Curvularia lunata* in an immunocompetent patient. *J. Clin. Microbiol*. 2004 Nov 1;42(11):5419-23.
10. Borreli L. Clarinet Player Who Didn't Clean Instrument For 30 Years Develops 'Saxophone Lung,' An Allergic Reaction To The Fungi Inside [Internet]. 2013. [cited July 7th, 2017]. Available from: <http://www.medicaldaily.com/clarinet-player-who-didnt-clean-instrument-30-years-develops-saxophone-lung-allergic-reaction-fungi>
11. Metzger F, Haccuria A, Reboux G, Nolard N, Dalphin JC, De Vuyst P. Hypersensitivity pneumonitis due to molds in a saxophone player. *Chest*. 2010 Sep 1;138(3):724-6.
12. Yanagihara M, Tachikawa N, Hanakawa H, Kawasaki M, Anzawa K, Ishizaki H, Mochizuki T, Udagawa SI, Sato Y. Tiny keratotic brown lesions on the interdigital web between the toes of a healthy man caused by *Curvularia* species infection and a review of cutaneous *Curvularia* infections. *Mycoscience*. 2010 May 1;51(3):224-33.
13. da Cunha KC, Sutton DA, Fothergill AW, Gené J, Cano J, Madrid H, de Hoog S, Crous PW, Guarro J. In vitro antifungal susceptibility and molecular identity of 99 clinical isolates of the opportunistic fungal genus *Curvularia*. *Diagn Microbiol Infect Dis*. 2013 Jun 30;76(2):168-74.
14. Madrid H, da Cunha KC, Gené J, Dijksterhuis J, Cano J, Sutton DA, Guarro J, Crous PW. Novel *Curvularia* species from clinical specimens. *Persoonia*. 2014 Dec;33:48.
15. Manamgoda DS, Cai L, McKenzie EH, Crous PW, Madrid H, Chukeatirote E, Shivas RG, Tan YP, Hyde KD. A phylogenetic and taxonomic re-evaluation of the *Bipolaris-Cochliobolus-Curvularia* complex. *Fungal Divers*. 2012 Sep 1:1-4.