**Case Report**

*Nocardia harenae*, an uncommon causative organism of mycetoma: report on two patients

Nicole S. Kresch-Tronik,1 Erika M. Carrillo-Casas,2 Roberto Arenas,3 Carlos Atoche,4 Luis A. Ochoa-Carrera,5 Juan Xicohtencatl-Cortes,6 Ángel H. Manjarrez-Hernández7 and Rigoberto Hernández-Castro5

**Correspondence**
Rigoberto Hernández-Castro
rigo37@gmail.com

1Servicio de Dermatología, Hospital General ‘Dr Manuel Gea González’, Tlalpan 14080, Mexico
2Departamento de Biología Molecular e Histocompatibilidad, Hospital General ‘Dr Manuel Gea González’, Tlalpan 14080, Mexico
3Departamento de Micología, Hospital General ‘Dr Manuel Gea González’, Tlalpan 14080, Mexico
4Departamento de Micología, Centro Dermatológico ‘Dr Fernando Latapi’, Mérida, Yucatán, Mexico
5Departamento de Ecología de Agentes Patógenos, Hospital General ‘Dr Manuel Gea González’, Tlalpan 14080, Mexico
6Departamento de Infectología, Hospital Infantil de México ‘Federico Gómez’, Mexico City, Mexico
7Departamento de Salud Pública, Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico City, Mexico

Mycetoma is the most frequently diagnosed deep mycosis in Mexico and is caused, in 86 % of cases, by *Nocardia brasiliensis*. Worldwide, *Nocardia harenae* has not been previously reported as a causative agent of human mycetoma. Herein we report, to our knowledge, the first two human cases of mycetoma due to *N. harenae* in a clinical setting. The strains were identified by phenotypic and molecular techniques. Both cases were characterized by long-lasting mycetoma that had previously been failed to be cured and had shown resistance to therapy. However, in our hospital, a multidrug therapy proved to be effective in these cases.

**Introduction**

Mycetoma is a late-stage infection, characterized by a chronic localized indurate, and is a slowly progressive and often painless cutaneous or subcutaneous disease. Usually, hands, feet, legs or arms are involved and macroscopically visible grains of various sizes and colours often are considered pathognomonic. Mycetoma is a globally distributed disease and is considered endemic in tropical and subtropical regions. In Mexico, 86 % of cases are caused by *Nocardia brasiliensis* (Ameen & Arenas, 2009; López-Martínez *et al.*, 1992). Members of the genus *Nocardia* are found in soil, dust, sand, decaying vegetable matter and aquatic environments (Fahal, 2004). The main route of acquisition is through direct inhalation of contaminated particles or by direct inoculation through the skin (Brown-Elliott *et al.*, 2006).

The introduction of molecular methods for use in the identification of new *Nocardia* species in various pathologies and habitats led to the description of *Nocardia harenae* in 2006, the type strain of which was isolated from beach sand on the coast of Jeju Island, South Korea, and classified based on its 16S rRNA gene sequence. Based on 16S rRNA analysis, *N. harenae* WS-26T was most closely related to *Nocardia carneae* DSM 4339T, *Nocardia cyriaci-georgica* DSM 44484T, *Nocardia flavourosea* JCM 3332T, *Nocardia pigrifrangens* 7031T, *Nocardia sienata* IFM 10088T and *Nocardia testacea* IFM 0937T (Seo & Lee, 2006). To our knowledge, no clinical cases have been reported or associated with this species since it was first identified. We believe this to be the first report of *N. harenae* obtained from a clinical case in the Mexican Caribbean.

**Case one**

The first case was a 63-year-old female, native to Campeche, Mexico, who had a 20-year history of multiple small sinus tracts, local inflammation and purulent discharge from her right thigh and knee (Fig. 1). On examination at the ‘Dr Fernando Latapi’ Dermatological Center, Yucatan, Mexico, the patient presented with anaemia and leukopenia. She was treated with iron, folic acid and an immunostimulant (Ismigen); no liver disorders were detected. Previously, the patient had received multiple treatments, which included...
use of the following antibiotics: trimethoprim–sulfamethoxazole and dapsone, clofazimine, rifampicin, amikacin, gatifloxacin, and amoxicillin/clavulanic acid.

Direct examination showed small multilobular grains of *Nocardia* (100–200 μm). Culture on Sabouraud dextrose agar showed chalky growth with tiny hyphae, which tested positive for acid-fast filaments using Kinyoun stain. No sensitivity tests were performed. The isolate was identified as *N. harenai* strain 441. The recommended treatment included intravenous injections of amikacin (1 mg kg⁻¹) and imipenem (500 mg) three times a day for 8 days and partial remission was observed with six lesions persisting. Follow-up treatment with trimethoprim/sulfamethoxazole (160/800 mg day⁻¹), dapsone (100 mg day⁻¹) and mynociclin (100 mg day⁻¹) was recommended for the next 2 years until the patient was clinically cured.

**Case two**

The second case was a 34-year-old male, native to Ucu, Yucatan, Mexico, who was first examined at the ‘Dr. Fernando Latapi’ Dermatological Center, Yucatan, Mexico. The patient presented small nodular lesions with no secretion on his left forearm (Fig. 2) with a 1 year history of asymptomatic sinus tracts. Direct examination showed small multilobular sinus tracts. Direct examination showed small multilobular grains. Culture on Sabouraud dextrose agar showed white colonies with tiny hyphae, which tested positive for acid-fast filaments using Kinyoun stain. No susceptibility tests were performed. The isolate was identified as *N. harenai* strain 21. Treatment comprised a multidrug therapy of trimethoprim/sulfamethoxazole (800/160 mg day⁻¹) and dapsone (100 mg day⁻¹) for 10 months. This resulted in complete remission, which was confirmed after 2 years of follow-up examinations.

Molecular identification of isolates from both cases was achieved by 16S rRNA gene sequencing, using a set of primers (5’-GGATCCTTTTGATCCTGGCTCAGGAC-3’ and 5’-ACTTGACGTCGTCCCCACCTTCCT-3’) that were designed based on the 16S rRNA gene sequence of *Nocardia wallacei* ATCC 49872, formerly *Nocardia asteroides* (accession number AY191251), to amplify a PCR product of 1120 bp. The PCR fragments were purified and sequenced in both directions. The sequences displayed 100% identity with *N. harenai* CDC <USA-GA> W9742 (acc no. GQ376170) and *N. harenai* strain WS-26T (acc no. DQ282122); and 97% identity with *Nocardia transvalensis* N630 (acc no. Z82240.1) and *Nocardia asiatica* CDC <USA-GA> W8323 (acc no. GQ217495). The complete 16S rRNA gene sequences determined for both *N. harenai* strains 441 and 21 have been deposited in GenBank under accession numbers HQ896358 and JF264836, respectively.

**Discussion**

Many *Nocardia* species have been implicated in human infections but the geographical prevalence of each species throughout the world may differ dramatically and some are uncommon. The cases reported herein are interesting because they are believed to be the first cases of mycetoma due to *N. harenai* in a country where *N. brasiliensis* is the prevalent aetiologic agent of mycetoma. *N. harenai* was first characterized by Seo & Lee (2006) as a novel species that differed from the previously described members of the genus *Nocardia* by its molecular and biochemical characteristics.

In the present cases, the direct examination of the grains, followed by determination of phenotypic characteristics of colonies in Sabouraud culture (Muñoz et al., 2007), suggested...
probable cases of *N. brasiliensis* infection but further examination using molecular techniques allowed the precise identification of *N. harenae* as the aetiological agent. Nowadays, the development of specific molecular techniques makes it possible to accurately identify species capable of infecting humans and, combined with conventional methods, these tools proved useful in obtaining an accurate *Nocardia* identification (Brown-Elliott et al., 2006; Roth et al., 2003; Conville et al., 2010). 16S rRNA gene sequencing has been used for identification at the species level, resulting in a considerable reclassification of members of the genus *Nocardia*, and, due to their distinctive and unique sequence arrangements, it is considered the gold standard for identification of these organisms (Roth et al., 2003; Conville et al., 2010).

Clinical experience and *in vitro* antibiotic susceptibility analysis have shown that the management of *Nocardia* infections must be individualized. *Nocardia* actinomycetoma is usually managed with an empirical therapy. For patients with local or disseminated disease, a three-drug regimen consisting of trimethoprim/sulfamethoxazole, amikacin, and either ceftriaxone or imipenem is preferred because using these drugs in combination prevents drug resistance, eradicates any residual infection and covers all clinical isolates (Brown-Elliott et al., 2006). Remarkably, the chosen treatment of a carbapenem with broad microbicidal activity confirmed our previous observations in that imipenem showed an impressive clinical response (Fuentes et al., 2006; Ameen et al., 2010, 2011).

The experience described herein will lead to the improvement of patient care and facilitate research into the pathogenesis and epidemiology of *Nocardia*-related disease. However, further studies are required to validate these observations.

In these cases, we used molecular methods to accurately identify the causative agent, leading to better case management and treatment. The total remission in both of these cases proved that the correct course of treatment was chosen.

### References


