Tropical medicine rounds

Isolation of both *Pseudozyma aphidis* and *Nocardia otitidiscaviarum* from a mycetoma on the leg

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Abstract
We describe a case of mycetoma which typified the classic presentation of the disease: a male farmer with affection of the lower limbs and a history of trauma. The patient presented with a swollen right lower limb showing multiple discharging sinuses for 25 years. Histopathologically, grains were found by HE stain, and clustered yeast-like cells were observed by PAS stain. The distinctive ‘dot-in-circle’ sign was found through MRI. Besides *Nocardia otitidiscaviarum*, *Pseudozyma aphidis* was isolated from deep tissue culture, and the identification of the etiologic species was ascertained by DNA sequencing.

Generally speaking, *Nocardia otitidiscaviarum* is an infrequent cause of mycetoma, and *Pseudozyma* species are usually isolated from plant material rather than clinical specimens. This is the first case of mycetoma from which both *Nocardia otitidiscaviarum* and *Pseudozyma aphidis* were isolated.

Introduction
Mycetoma is a chronic, granulomatous, subcutaneous, inflammatory disease endemic in tropical and subtropical regions of the world. About 60% of cases are caused by actinomycetes, while the rest are caused by true fungi. It is hard to ascertain the infecting organisms because some grow slowly, and usually cultures should be held for 3–4 weeks. Morphological identification is often difficult. Advanced molecular technology such as sequence analysis is promising for the rapid and accurate identification of pathogens.

The genus *Nocardia* belongs to the family of *Nocardiaceae*, which forms a homogeneous cluster among the Corynebacterineae, a suborder of the order Actinomycetales, mainly known as an opportunistic pathogen in immunocompromised individuals.

*Nocardia otitidiscaviarum* was formerly called *Nocardia caviae*, which is an infrequent cause of mycetoma. The yeast-like fungus *Pseudozyma* belongs to Ustilaginales, which is usually isolated from plant material rather than clinical specimens.

Case report
A 51-year-old male farmer presented with multiple discharging sinuses over a swollen right lower limb for 25 years but with no underlying debilitating disease. Initially, a single, firm, painless nodule on the dorsal surface of the foot had appeared after injury. Several years later, multiple nodules developed and gradually spread to the leg. Some nodules ruptured, forming sinuses discharging a yellowish material. The clinical symptoms had improved after antifungal monotherapy 10 years ago, but he failed to receive sustained treatment due to economic reasons.

On physical examination, an irregular swelling from the popliteal fossa down to the toes was seen. The skin was hyperpigmented with numerous sinuses discharging yellowish pus (Fig. 1). However, there were no grains visible to the naked eye from any of the discharging sinuses.

Abnormal laboratory results included an elevated leukocyte count of $20.1 \times 10^9/l$ (68% neutrophils), C-reactive protein of 78 mg/l, and erythrocyte sedimentation rate of 64 mm/h. The blood serum levels of IgA, IgG, IgM, C3, and C4 were normal. Tests for antinuclear antibodies, rheumatoid factor, and HIV antibody were negative.

A magnetic resonance image (MRI) of the affected limb was performed to characterize and evaluate the extent of the disease. T2-weighted MRI revealed extensive inflammatory change with multiple focal fluid collections. Some of these lesions showed a central tiny hypointense focus, resulting in the dot-in-circle sign (Fig. 2).
Deep incisional biopsies from the dorsum of the left foot were sent for histopathologic and microbiologic examinations. Hematoxylin and eosin staining revealed grains that were surrounded by inflammatory cells (Fig. 3a), and periodic acid-Schiff stain showed clustered yeast-like cells (Fig. 3b).

Pus direct microscopic examination together with pus and tissue culture (aerobic and anaerobic) were performed. After one week of incubation at 37 °C, aerobic culture of the tissue on blood agar yielded yellow–white colonies, 1–2 mm in diameter. A gram stain of the colonies showed gram-positive, branching, filamentous bacilli, which were weak positive to acid-fast stain. Thus, a preliminary identification of *Nocardia* spp. was made. The cultured material was further identified by polymerase chain reaction (Beijing Sunbiotech Co. Ltd, China). DNA sequencing of the 16S ribosomal RNA gave a positive BLAST result for *Nocardia otitidiscaviarum*. After four weeks of incubation at 25 °C in ambient air, tan-yellow, wrinkled, and moist yeast-like colonies were yielded on Sabouraud glucose agar. Wet mount showed septate, hyaline hyphae. Identification of the fungus was attempted by using a commercial yeast identification kit (Yeast Biology Card; VITEK; bioMerieux, Inc). The organism was positive for urea hydrolysis and for assimilation of galactose, lactose, sucrose, maltose, xylose, arabinose, trehalose, melezitose, raffinose, glycerol, glucitol, glucose, and nitrate, and was negative for assimilation of cellobiose and dulcitol. The morphological and biochemical features suggested that the organism was yeast of the order Ustilaginales. The fungus-specific universal primers internal transcribed spacer 1 (ITS1) and internal transcribed spacer 4 (ITS4) were used to amplify the internal transcribed spacer and 5.8S regions of fungal ribosomal genes. The nucleotide sequence showed 99% similarity to *Pseudozyma aphidis* with accession numbers AF294699.1, AB204896.1, and EU105207.1 (http://www.ncbi.nlm.nih.gov).

The patient was treated with trimethoprim/sulfamethoxazole 160/800 mg orally twice daily and itraconazole 100 mg orally twice daily along with repeated debridement.

Figure 1  Mycetoma of the right leg before treatment: inflammatory nodules, fistulas, and abscesses on the left leg. (a) Overview; (b) detail

Figure 2  T2-weighted magnetic resonance image of the right leg reveals extensive inflammatory soft tissue changes with fluid collections and chronic fibrous degeneration. T2-weighted magnetic resonance image of the right foot reveals multiple small spherical hyperintense lesions separated by tissue of low signal intensity. Some of these lesions show a central tiny hypointense focus, resulting in the dot-in-circle sign

Figure 3  (a) Histological examination of a skin lesion biopsy, showing a heart-shaped grain with an eosinophilic rim presented in the background of an inflammatory infiltrate (hematoxylin and eosin stain, ×100). (b) Periodic acid-Schiff-positive clustered yeast-like cells were observed (periodic acid-Schiff stain, ×400)
Within one week, the swelling and draining improved slightly. The sinuses stopped draining after two months' therapy and swelling subsided.

After one year's treatment, the lesions healed without relapse and with scarring. Itraconazole was well tolerated, and no adverse events occurred during therapy.

**Discussion**

There are four clinical types of cutaneous nocardiosis: mycetoma, lymphocutaneous infection, supplicative skin infection, and secondary cutaneous infection with disseminated disease. Mycetoma is a late-stage infection as in the case presented here. *Nocardia otitidiscaviarum* is less pathogenic than other species of *Nocardia*, being responsible for 2.9% of cases of infections due to *Nocardia* in the United States. The microbiological features of nocardiosis in Spain, Greece, Saudi Arabia, India, Thailand, and Japan are shown in Table 1.

In 1995, Clark et al. reviewed 28 cases of primary cutaneous nocardiosis caused by *N. otitidiscaviarum*, among these were four cases of mycetoma with complete clinical information. From a retrospective study of 264 cases of mycetoma in West Bengal, the incidence of *N. otitidiscaviarum* infections was 14.4% (n = 38). During the past 10 years, six new cases of cutaneous nocardiosis have been reported in English, and the clinical features, therapy, and outcome of these patients are summarized in Table 2.

In a review of medical literature, there have been only a few sporadic cases concerning *Pseudozyma* spp. and *Ustilago* spp. In 2003, Suglta et al. first isolated *Pseudozyma* spp. from patients' blood, but these patients' clinical characteristics were not presented. In 2008, Shau-Shau Lin et al. first reported a child case of central venous catheter infection associated with *Pseudozyma aphidis*. In relation to skin infections, one patient had a persistent skin rash manifesting as scaly erythematous plaques on the right chest wall, left knee, and over the nasal alae and philtrum. Fungal scrape and culture of the scaly lesions over the nasal alae and philtrum was positive for *Ustilago* spp. Although the pathogenesis of this heterobasidiomycetous yeast is unclear, *Ustilago maydis* has become a highly attractive model in addressing fundamental questions about fungal pathogenesis. The initiation of pathogenic development is characterized by a morphological transition (dimorphic switch) from yeast-like budding cells to tip-growing hyphae. Insights into molecular mechanisms of complex signaling pathways involved in the morphological transition and genes related to pathogenic development could help to determine the pathogenic mechanisms of *Ustilagomaydis* and other related yeasts, such as *Pseudozyma* spp.

Treatment of mycetoma depends on the causative organism. Individuals infected with eumycetes require both medical and surgical intervention. The newer azoles, voriconazole and posaconazole, offer the promise of improved therapy. In contrast with eumycetes, infections caused by actinomycetes have a better response to medical management with antibacterials. The mainstay of antimicrobial therapy of nocardial infection has been sulfonamide combination. Other medications that have been used with efficacy against certain actinomycetes include dapsone, amikacin, and imipenem. A combination therapy of amikacin, cotrimoxazole, and rifampicin has been proven effective for treatment of resistant cases of actinomycetoma.

Our case is noteworthy since it appears to be the first reported case of mycetoma associated with *Nocardia otitidiscaviarum* and *Pseudozyma aphidis*. More than five specimens were sent for culture. Repeated cultures of pus were positive for *Nocardia otitidiscaviarum*, and one deep tissue culture was positive for *Pseudozyma aphidis*. Although a positive result was only attained in one culture, the possibility of contamination of deep tissue culture is quite low, and the odds of contamination of

<table>
<thead>
<tr>
<th>References</th>
<th>Country</th>
<th>Year</th>
<th>Total <em>Nocardia</em> spp.</th>
<th>Total <em>N. otitidiscaviarum</em></th>
<th>Total cutaneous <em>Nocardia</em> spp.</th>
<th>Total cutaneous <em>N. otitidiscaviarum</em></th>
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<tbody>
<tr>
<td>6</td>
<td>Madrid, Spain</td>
<td>1995–2006</td>
<td>43</td>
<td>4</td>
<td>3</td>
<td>1</td>
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<td>7</td>
<td>Barcelona, Spain</td>
<td>1997–2003</td>
<td>27</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Crete, Greece</td>
<td>2003–2007</td>
<td>15</td>
<td>4</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>Riyadh, Saudi Arabia</td>
<td>1987–2003</td>
<td>19</td>
<td>4</td>
<td>2</td>
<td>1</td>
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<tr>
<td>10</td>
<td>Chandigarh, India</td>
<td>2004–2006</td>
<td>12</td>
<td>1</td>
<td>2</td>
<td>1</td>
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<tr>
<td>11</td>
<td>Nonthaburi, Thailand</td>
<td>1996–2003</td>
<td>96</td>
<td>Not available</td>
<td>Not available</td>
<td>Not available</td>
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<tr>
<td>12</td>
<td>Chiba, Japan</td>
<td>1992–2001</td>
<td>303</td>
<td>14</td>
<td>84</td>
<td>6</td>
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Table 2 Review of clinical features, therapy and outcome of cutaneous nocardiosis

<table>
<thead>
<tr>
<th>References</th>
<th>Year of publication</th>
<th>Country of residence/origin</th>
<th>Age/sex</th>
<th>Site</th>
<th>Disease duration</th>
<th>Diagnosis</th>
<th>History of trauma</th>
<th>Underlying conditions</th>
<th>Main treatment</th>
<th>Course of treatment</th>
<th>Outcome</th>
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<tr>
<td>18</td>
<td>2001</td>
<td>United Arab Emirates/Oman</td>
<td>50/M</td>
<td>Right hand</td>
<td>10 years</td>
<td>Cutaneous nocardiosis, actinomycetoma</td>
<td>NA</td>
<td>NA</td>
<td>Amikacin IV, Rifampicin PO, TMP-SMZ PO</td>
<td>4 weeks × 3</td>
<td>52 months</td>
</tr>
<tr>
<td>19</td>
<td>2002</td>
<td>American/NA (Caucasian)</td>
<td>77/M</td>
<td>Left leg</td>
<td>3–4 weeks</td>
<td>Primary lymphocutaneous infectious</td>
<td>Questionable for a recent spider bite</td>
<td>RA (mildly immunosuppressed)</td>
<td>Minocycline PO, Clarithromycin PO</td>
<td>NA</td>
<td>6 months</td>
</tr>
<tr>
<td>20</td>
<td>2002</td>
<td>Japan/Japan</td>
<td>69/F</td>
<td>Left hand</td>
<td>12 d</td>
<td>Primary lymphocutaneous nocardiosis</td>
<td>Yes</td>
<td>Immunocompetent</td>
<td>Incision drainage TMP-SMZ PO</td>
<td>–</td>
<td>6 months</td>
</tr>
<tr>
<td>21</td>
<td>2005</td>
<td>France/NA (Caucasian)</td>
<td>70/M</td>
<td>Right forearm</td>
<td>2 weeks</td>
<td>Primary cutaneous infections</td>
<td>Yes</td>
<td>RA (receiving infliximab), DM</td>
<td>Ofloxacin PO, Clindamycin PO</td>
<td>3 months</td>
<td>3 months</td>
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<tr>
<td>22</td>
<td>2007</td>
<td>Germany/Turkey</td>
<td>55/M</td>
<td>Left leg</td>
<td>20 years</td>
<td>Cutaneous abscesses</td>
<td>NA</td>
<td>Immunocompetent</td>
<td>Amikacin IV, Imipenem IV, TMP-SMZ PO</td>
<td>4 weeks</td>
<td>4 weeks over 4 years</td>
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<tr>
<td>23</td>
<td>2009</td>
<td>Brazil/African-American</td>
<td>37/M</td>
<td>Right hand</td>
<td>4 years</td>
<td>Mycetoma</td>
<td>Yes</td>
<td>NA</td>
<td>TMP-SMZ PO</td>
<td>10 months</td>
<td>Clinical improvement without relapse</td>
</tr>
</tbody>
</table>

DM, diabetes mellitus; IV, intravenous; NA, not available; PO, per os; RA, rheumatoid arthritis, TMP-SMZ: trimethoprim-sulfamethoxazole.
such a scarce fungus is unlikely. The identification of the etiologic species was established by its typical morphologic characteristics and DNA sequencing. Based on the clinical, radiologic, histopathologic, and microbiologic findings, the diagnosis of mycetoma caused by *Nocardia otitidiscaviarum* could be established. Meanwhile, we believe fungal infection played an important role in our case for the following reasons: (1) clustered yeast-like cells were observed by periodic acid-Schiff staining; (2) deep tissue culture was positive for *Pseudozyma aphidis*; (3) the patient responded to single antifungal therapy. The long course of the disease made it difficult to decide whether *Pseudozyma aphidis* was a primary or secondary agent. *Nocardia* species are well known pathogenic agents of mycetoma. We consider that *Pseudozyma aphidis* was a secondary agent. Nevertheless, the possibility of concurrent infection, even the major role of *Pseudozyma aphidis* in the pathogenesis of our case, could not be ruled out. Isolation of both *Nocardia otitidiscaviarum* and *Pseudozyma aphidis* from the mycetoma on the patient’s leg made this case different.

References


