CASE REPORT

Actinomycetoma caused by *Nocardia otitidiscaviarum*: Report of a case in Taiwan with long-term follow-up

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A B S T R A C T

Actinomycetoma is a chronic granulomatous infection, with *Nocardia* species being one of the infecting pathogens. Infections caused by *N. otitidiscaviarum* are relatively rare compared with those caused by other *Nocardia* species. Conventional methods for the diagnosis of nocardiosis based on phenotypic characterization of the strains (e.g., morphology, histopathology) are relatively time consuming and nonspecific. Molecular techniques have become the better choice for prompt and accurate identification of *Nocardia* isolates. We report a case of actinomycetoma due to *N. otitidiscaviarum* characterized by 16S ribosomal RNA (16S rRNA) gene sequence analysis and the long-term follow-up.

Introduction

Actinomycetoma is a chronic granulomatous infection of the skin, subcutaneous tissue, fascia, and bone caused by traumatic inoculation of actinomycetes, with the feet being the predominant site. *Nocardia* species are one of the infecting pathogens that cause actinomycetoma. Primary cutaneous nocardiosis has three main clinical manifestations: superficial skin infections, mycetoma, and sporotrichoid lymphocutaneous disease. More than 50 species in the genus *Nocardia* have currently been characterized by phenotypic and molecular methods; however, nocardiosis caused by *N. otitidiscaviarum* is relatively rare compared with infections caused by other species. Herein, we report the case of a 71-year-old man with lymphocutaneous actinomycetoma on the left calf that started as mycetoma at the ankle and 30 years of follow-up. The developments of different diagnostic methods are also discussed.

Case report

A 71-year-old male coal miner had multiple erythematous indurated nodules, abscesses, and granulation tissue on the left lower leg for nearly 30 years. He was initially seen in October 1982 with a 2-year history of multiple erythematous nodules and pus formation over the left ankle with no obvious history of trauma (Figure 1A). Mycetoma was diagnosed based on the clinical manifestations and histopathologic finding of a typical sulfur granule on a specimen taken from the left ankle (Figure 1B). Because no microorganisms were isolated from numerous bacterial and fungal cultures, various antibiotic and antifungal agents, including penicillin, oxacillin, doxycycline, sulfamethoxazole–trimethoprim (cotrimoxazole), gentamicin, griseofulvin, and ketoconazole, were prescribed sequentially. However, the lesions persisted.

The patient was lost to follow-up for 14 years; however, he returned in 2007 because of worsening skin lesions. The lesions continued to increase in number and spread upward from the left ankle to the left popliteal fossa in a sporotrichoid pattern over the next 3 years, resulting in multiple erythematous protuberant nodules of granulation tissue and abscesses with purulent discharge. The previous ankle lesion healed, leaving a whitish scar (Figure 2A). Moreover, he had fixed drug eruptions after repeated use of cotrimoxazole. Subacute cutaneous lupus erythematosus presented as erythematous annular macules over sun-exposed areas for 3 months starting in September 2008. This was confirmed by pathology and positive antinuclear antibody (1:320, speckled) and anti-SSA (>240 U/mL)/SSB (232 U/mL) antibodies, although antidouble-stranded DNA and anti-Smith antibodies were negative. The lupus was controlled using low dose oral prednisolone and hydroxychloroquine.
Repeat laboratory studies revealed normocytic anemia (hemoglobin, 10.5 g/dL; mean corpuscular volume, 85.8 fl) and a slightly elevated erythrocyte sedimentation rate (54 mm/h; normal, 0–20 mm/h) with normal flow cytometry analysis of T lymphocytes, implying a chronic inflammatory process. Magnetic resonance imaging (MRI) of the left lower extremity disclosed extensive subcutaneous swelling in the left lower leg and pyomyositis without bony destruction. Histopathology of a specimen from one protuberant nodule showed ulcers covered with necrotic dermis and diffuse dermal and subcutaneous fibrosis with acute and chronic inflammation and scattered microabscesses. One sulfur granule was found in a microabscess.

Figure 1  (A) Initial presentation of multiple erythematous nodules with pus formation over the left ankle in October 1982; (B) histopathology of a section of the nodule showed one sulfur granule (arrow) surrounded by inflammatory cells present in the deep dermis (hematoxylin-eosin stain, ×100).

Figure 2  (A) Skin lesions on the left lower leg in March 2010 that indicated multiple nodules with proximal purulent discharge and previously healed skin lesions on the left ankle; (B) histopathology revealed ulcers with necrotic dermis, diffuse fibrosis with inflammation, and scattered microabscesses (hematoxylin-eosin stain, ×20). One sulfur granule was found in one microabscess (hematoxylin-eosin stain, ×400); (C) after 9 days of culture, chalky-white colonies with rough and verrucous surface grew on the blood agar side of the blood/MacConkey agar biplate, suggestive of Nocardia species.
under the ulcer (Figure 2B). Filamentous structures in the sulfur granule were partially acid-fast on Fite stain and negative on Kinyoun acid-fast stain. Periodic acid-Schiff staining was also negative.

Nine-day cultures of native lesion tissue grew chalky-white colonies with a rough and verrucous-like surface, indicative of Nocardia species on the blood agar of a blood/MacConkey agar biplate (Figure 2C). Identification of the species done at the Mycotic Diseases Laboratory of the Centers for Disease Control in Taiwan revealed that the colonies were able to hydrolyze both hypoxanthine and xanthine. To further identify the species, the 16S ribosomal RNA gene of the clinical isolate 04-901-553856 was sequenced as well as the Institute of Food Microbiology (IFM) reference strains (now the Research Center of Pathogenic Fungi and Microbial Toxicoses, Chiba University). The sequence of Nocardia brasiliensis DSM43758 was obtained from the National Center for Biotechnology Information (NCBI) database. Sequences were aligned using Clustal X (version 2.0.12) and a phylogenetic tree was generated using the neighbor-joining method by MEGA (version 5.05). Bootstrap analysis with 1000 replications was performed. The 16S ribosomal RNA sequence of the isolate 04-901-553856 was found to cluster with that of a Nocardia otitidiscaviarum reference strain (IFM0239), with a bootstrap value of 82% (Figure 3).

An antimicrobial susceptibility test revealed that the isolate was susceptible to amikacin, gentamicin, tetracycline, meropenem, imipenem, and cotrimoxazole. After treatment failed with oral tetracycline and doxycycline, intravenous amikacin (400 mg twice daily) and imipenem—cilastatin (500 mg four times per day) were started together with topical tetracycline (Figure 2B). Filamentous structures in the sulfur granule were partially acid-fast on Fite stain and negative on Kinyoun acid-fast stain. Periodic acid-Schiff staining was also negative.

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Many serologic tests including immunoassays and enzyme-linked immunosorbent assays that detect circulating antibodies have been developed for the early diagnosis of nocardial disease. However, using a single serologic method for the detection of nocardial infections may be limited due to the numerous species causing infections and the potential lack of sensitivity in immunocompromised patients. Molecular techniques such as multi-locus sequence analysis of gyrB of the β subunit of DNA topoisomerase (gyrB), 16S rRNA (16S), subunit A of SecA preprotein locus sequence analysis of gyrase B of the gyrB, topoisomerase (gyrB), 16S rRNA (16S), subunit A of SecA preprotein (secA1), the 65-kDa heat shock protein (hsp65), and RNA polymerase (rpoB) have been developed to identify Nocardia species. Among these, 16S rRNA gene sequence analysis, which was developed in the 1980s for bacterial identification, is an excellent and extensively used technique to identify Nocardia isolates at the species level. In the present case report, the 16S rRNA phylogenetic tree revealed that Nocardia isolates (number 04-901-553856) from the patient were clustered with the reference strain of N. otitidiscaviarum (strain number IFM0239).

Before the availability of antibiotic susceptibility of Nocardia species, empirical antibiotics with cotrimoxazole or minocycline were the most frequently used treatment for localized or mild nocardiosis, while combination therapy with a carbapenem or a third-generation cephalosporin with or without amikacin was recommended in severe cases or for those with central nervous system involvement. For those who fail to respond to standard antibiotic therapy, successful treatment with linezolid and amoxicillin-clavulanate has been reported. The mean treatment duration with amoxicillin–clavulanate has been reported to be 9.6 months, with a minimum of 4 months and a maximum of 22 months. However, geographic variations and species distribution have a significant impact on differences in terms of antibiotic susceptibility.

In Taiwan, Lai et al found that eight N. otitidiscaviarum isolates were susceptible to linezolid, sulfamethoxazole, and amikacin, but not to amoxicillin–clavulanate, ceftriaxone, imipenem, or ciprofloxacin. Co-trimoxazole (sulfamethoxazole-trimethoprim) is the most common choice for treating actinomycetoma caused by Nocardia otitidiscaviarum. The total treatment duration regardless of antibiotic regimen is at least 10 months and as long as 55 months (Table 1). In the present case report, the drug of choice was based on the antimicrobial susceptibility test and the patient’s past history of fixed drug eruptions from co-trimoxazole.

In our case, the prolonged clinical course and unsatisfactory therapeutic efficacy may be due to several factors, including the accuracy of the initial diagnosis, an immunocompromised status due to old age, subacute cutaneous lupus erythematosus, intolerable adverse events of antimicrobials leading to poor drug compliance, and frequent switching. Prompt diagnosis with genotypic methods and accurate antimicrobial susceptibility tests are useful and important in clinical practice.

In conclusion, herein we report a case that highlights the clinical course of actinomycetoma under various treatments for almost 30 years, with a partial response. Identification of the microbial isolates with molecular techniques, the selection of antimicrobials according to antimicrobial susceptibility tests, and compliance for complete treatment course are crucial for a good therapeutic response. To date, this patient is the first reported case of actinomycetoma induced by N. otitidiscaviarum with long-term follow-up in Taiwan.

Table 1 Review of the clinical features, therapy, and outcome of actinomycetoma caused by Nocardia otitidiscaviarum.

| Author             | Age/sex | Location          | Disease duration (yr) | History of trauma | Underlying disease | Treatment/duration | Outcome          |
|--------------------|---------|-------------------|-----------------------|-------------------|--------------------|--------------------|-------------------|------------------|
| Sandhu et al       | 20/M    | Right knee        | 2                     | No                | NA                 | NA                 | Isoniazid + TMP-SMZ >1 yr | Improvement |
| Alteras et al      | 41/M    | Left foot         | 2                     | NA                | NA                 | NA                 | Dapsone + TMP-SMZ, po/1 yr | Improvement |
| Alteras et al      | 39/F    | Right foot        | ≥1                    | No                | NA                 | NA                 | Dapsone + TMP-SMZ, po/1 yr | Fistulas healed and no further “grains” were observed |
| Saül et al         | 54/M    | Left leg          | NA                    | Yes               | NA                 | NA                 | Dapsone + TMP-SMZ, po/NA  | Flattening of the lesions and no “grains” found by direct examination |
| Saarinen et al     | 46/M    | Right supra-clavicular region | 3           | No                | NA                 | NA                 | Dapsone + TMP-SMZ, po/NA  | Flattening of the lesions |
| Bonifaz et al      | 50/M    | Right hand, right armpit, chest wall | 10            | NA                | NA                 | NA                 | Amikacin (IV) / 3 weeks × 3 Rifampicin, po + TMP-SMZ, po/52 mos | Cured |
| Magalhães et al    | 52/M    | Lumbar back       | 6                     | NA                | NA                 | NA                 | Amoxicillin-clavulanate, po/6 mos | Cured |
| Chen et al         | 37/M    | Right hand        | 4                     | Yes               | None               | NA                 | TMP-SMZ, po/10 mos         | Improvement |
| Present case       | 51/M    | Right lower leg   | 25                    | Yes               | None               | NA                 | TMP-SMZ, po/1yr            | Healed without relapse |
| Present case       | 71/M    | Left lower leg    | ~30                   | No                | SCLE               | NA                 | Amikacin + imipenem-claflastatin; then Amikacin + meropenem (IV)/total 3 wks TMP-SMZ, po/10 d | Skin lesions diminished in size with less discharge |

d = day; IV = intravenous; M = male; mo = month; NA = not available; PO = per os (by way of the mouth); SCLE = sub-acute cutaneous lupus erythematosus; yr = year.

References


