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Case Report: Mycetoma Caused by *Nocardia yamanashiensis*, Papua New Guinea

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Abstract. We report the first documented case of a mycetoma caused by *Nocardia yamanashiensis* after the initial description of this species. The 16S-rRNA gene sequence analysis was used to identify the novel species, which showed a similarity of 99.9% to the gene sequence of the type strain. The case showed both clinical non-response and reduced susceptibility *in vitro* to amoxicillin plus clavulanate, and it was treated successfully with trimethoprim-sulfamethoxazole and doxycycline. Given antibiotic resistance concerns, we suggest that antimicrobial susceptibility testing should be done for the majority of *Nocardia* species without well-established resistance patterns.

INTRODUCTION

Nocardia yamanashiensis was first isolated from the skin abscess of a 30-year-old female Japanese patient in 1987, but only in 2004 did Kageyama and others[1] establish its taxonomic position and reliable criteria for its identification on the basis of phenotypic and phylogenetic characters. Their study of this actinomycete through a 16S ribosomal DNA (rDNA) technique revealed that *N. yamanashiensis* is closely affiliated to *Nocardia pseudobrasiliensis* and *Nocardia otitidiscaviarum*. To our knowledge, there have not been subsequent reports of human infection caused by *N. yamanashiensis*.

Treatment of primary cutaneous *Nocardia* infection is often challenging and depends mainly on the susceptibility pattern of the causative species and severity of disease. There are currently more than 30 species of nocardiae of human clinical significance, but mycetomas are mostly produced by *Nocardia brasiliensis*, which is isolated from about 80% of cases.[2–4] Medical therapy with prolonged courses of antimicrobials is associated with substantial clinical improvement frequently observed within the initial 3 months of initiation of treatment. The most common antimicrobials used for this condition are sulfa drugs (sulfamethoxazole/trimethoprim and dapsone), aminoglycosides (streptomycin, amikacin), β-lactams (amoxicillin-clavulanate), and tetracyclines (minocycline). Combined drug therapy is always preferred to avoid drug resistance and to achieve microbiologic cure.

The study. We report a case of *N. yamanashiensis* mycetoma in a 34-year-old immunocompetent male. In January 2011, he was admitted to Lihir Medical Center (Lihir Island, Papua New Guinea) with subcutaneous and bone involvement of his right lower extremity. He lived in an impoverished rural area and reported a history of local trauma associated with dirt contamination of the wound 6 months previously.

The condition started as a single, small subcutaneous nodule and histological examination of the former lesion revealed focal epidermal hyperplasia with a chronic inflammatory infiltrate comprising neutrophils and lymphohistiocytes. Special stains for infective organisms, including Gram-positive bacteria, mycobacteria (i.e., modified acid-fast stain), *Leishmania*, fungi, and spirochetes were all negative. Despite 3 months of empirically prescribed antimicrobial treatment with a combination of high dose oral amoxicillin and clavulanate and surgical debridement at a peripheral center the lesion progressively worsened. On admission, the man presented with a firm and nontender severe swelling and deformity on the anteromedial aspect of his foot with multiple sinus tracts that opened to the surface and drained purulent material with granules (Figure 1). Standard x-ray studies revealed periosteal erosion and osteoporosis. On ultrasonography the lesion showed multiple thick-walled cavities, without acoustic enhancement, with grains represented as fine echoes at the bottom of the cavities. The patient denied any associated systemic symptoms such as fever, weight loss, or malaise. As the swelling progressively increased, he developed difficulty in walking and was forced to cease his usual employment.

Gram stain of pus aspirated from a cystic lesion on the foot showed no bacteria and the direct acid-fast staining was also negative. The specimen was cultured on blood, McConkey, and anaerobic agar and held for 5 days with no growth but also inoculated after decontamination (3% NaOH) into liquid mycobacterial agar (BacT/ALERT, Biomerieux, France) at 32 and 37°C and a chocolate agar slope at 32°C. After 11 days growth of gram-positive branching organisms that were partially acid fast by the modified Kinyoun method (1% sulphuric acid decolorization) appeared on the chocolate slope and subsequently in the 32°C liquid mycobacterial medium. Species identification was done by means of 16S rRNA gene sequence analyses that were performed by the Mycobacteriology section, Queensland Health Pathology Services. Primer sets of 16S-F3 (5′-CAG GCC TAA CAC ATG CAA GT-3′)/16S-R3 (3′-GGG CGG WGT GTA CAA GGC-3′) were used. The DNA was amplified by polymerase chain reaction (PCR) and purification of the PCR product and sequencing performed. Sequences obtained were compared with the public database GenBank (http://www.ncbi.nlm.nih.gov/blast) using blast searches. The 16S rRNA gene sequence (1,414 bp) of the isolate showed a similarity of 99.9% to *N. yamanashiensis* sp. nov. IFM 0265^T. Phenotypic characteristics were also consistent with this identification.

Susceptibility testing of reported antibiotics was performed by the broth microdilution method using the Clinical and Laboratory Standard Institute (CLSI) criteria with Sensititre microtiter trays (Sensititre; Treck Diagnostics Systems, West Sussex, England).[5] Minimum inhibitory concentrations were recorded after 3 days incubation (Table 1). Amoxicillin-clavulanate in combination showed poor activity. The isolates were susceptible to trimethoprim-sulfamethoxazole and amikacin, but tobramycin showed a low level of activity. The strain was susceptible to minocycline.

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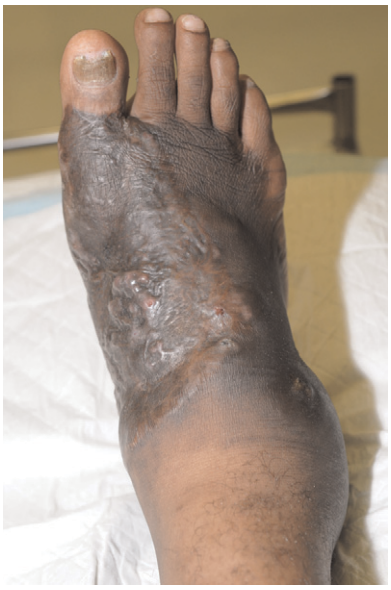

FIGURE 1. Primary cutaneous *Nocardia yamanashiensis* infection of a patient from Lihir Island, PNG, 2011. Note: A plaque of primary cutaneous *N. yamanashiensis* mycetoma on the anteromedial aspect of the foot and the right side swelling and sinus discharge. Source of photograph: Lihir Medical Center, Dr. Oriol Mitjà.

On the basis of the microbiological findings, our patient received a combination regimen of oral trimethoprim-sulfamethoxazole (TMP-SMX) (320 mg TMP and 1,600 mg SMX twice daily, respectively) combined with oral doxycycline (100 mg twice daily) for a period of 6 months, with a subsequent reduction in foot swelling, complete absence of discharge from sinuses, and healing of the papules and nodules.

CONCLUSIONS

This work describes the isolation of a bacteria identified as *N. yamanashiensis* as a cause of mycetoma in a patient in Papua New Guinea. This is a significant finding because there have been no reports of the isolation of this organism after the initial description of this species.[1] We are confident with the adequacy of the identification of this isolate because of the high similarity (99.9%) of the 16S gene sequence to that of the type strain. Conville and others[6] stated that nocardial isolates may not be identified correctly as *N. yamanashiensis* (and two other species) using the 16S rRNA gene when sequence analysis shows < 99.8% similarity, because the type strains of those species were found to have multiple differing copies of that gene. For clinical cases with a lower genetic similarity, an additional gene target could be tested to confirm the identification of an isolate as *N. Yamanashiensis.*

TABLE 1

Activities of antimicrobial agents against a clinical isolate of *Nocardia yamanashiensis* determined by the broth microdilution method (sensititre)

Antimicrobials	Minimum inhibitory concentration (MIC) (mg/L)	Susceptibility
Amox/clav	16/8	I
Imipenem	< 2.00	S
Trimethoprim/ Sulfamethoxazole	0.25/4.75	S
Amikacin	< 1.00	S
Tobramycin	> 16	R
Ciprofloxacin	> 4.00	R
Minocycline	< 1.00	S
Clarithromycin	< 0.06	S

Note: CLSI breakpoints/interpretations were used.[5]
I = intermediate; S = susceptible; R = resistant.

Managing *Nocardia* infections is often complicated by drug intolerance (e.g., manifested as cutaneous eruption following use of sulphonamides), treatment failure, recovery of primary drug-resistant strains, or development of resistance during therapy. Despite the described isolate was susceptible to agents that commonly cover *Nocardia* species (TMP-SMX and amikacin), the case was initially treated empirically for carbuncle with amoxicillin-clavulanate and progressed. This is not unexpected in mycetoma caused by *Nocardia*. Amoxicillin-clavulanate is an alternative treatment in *Nocardia* mycetoma in patients who cannot tolerate a sulphonamide.[7] The case we report demonstrated both clinical non-response and reduced susceptibility *in vitro* to amoxicillin-clavulanate. This has also been reported for mycetomas caused by other *Nocardia* species, such *Nocardia farcinica*, *N. otitidiscaviarum*, *Nocardia nova*, *N. pseudobrasiliensis*, and, *Nocardia mexicana*.[8–12] Therapeutic efficacy in individual patients may depend on species identity and on *in vitro* susceptibility studies. Therefore, for the majority of *Nocardia* species, that have not been studied for antimicrobial susceptibility, such as the newly described *N. yamanashiensis*, obtaining susceptibility testing would be prudent, especially when using an agent known to have variable activity against the genus.

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Disclosure: The patient provided signed consent authorizing publication of the case and images. Molecular identification was performed at the *Mycobacterium* Reference Laboratory, Queensland Health Scientific Services, Herston, Queensland, Australia.

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