Lung *Nocardia elegans* Infection Diagnosed on Matrix-assisted Laser Desorption Ionization-time of Flight Mass Spectrometry (MALDI-TOF MS)

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**Abstract**

A 73-year-old man with adult onset Still’s disease developed a high fever, coughing, dyspnea and bloody sputum and was therefore admitted to our hospital. Thoracic X-ray and CT scans revealed oval lesions in the bilateral lungs. A bacterial isolate from the sputum was identified to be *Nocardia elegans* (*N. elegans*) on comparative 16S rRNA gene sequencing and Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). The patient recovered following treatment with imipenem/cilastatin and amikacin. To the best of our knowledge, this is the first case of nocardiosis caused by *N. elegans* identified on MALDI-TOF MS.

**Key words:** *Nocardia elegans*, Still’s disease, Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS)

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**Introduction**

Nocardiosis is a potentially life-threatening infection caused by several species of the genus *Nocardia* (1). Most commonly, nocardiosis presents as a pulmonary disease. Members of the *Nocardia asteroides* complex primarily cause pulmonary disease, and, except for *Nocardia nova*, all of these microorganisms are prone to extrapolmonary dissemination (2-6).

The first case of *N. elegans* pulmonary infection was reported by Yassin et al. (7), with six cases having since been reported, including one case involving a cat (8). In this case report, we document the first case of pulmonary infection caused by *N. elegans* in a patient with adult-onset Still’s disease (AOSD) diagnosed based on the findings of morphological, physiological, chemotaxonomic and molecular biological methods, particularly Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS).

**Case Report**

A 73-year-old man with AOSD taking prednisolone and cyclosporine developed a high fever, with a frequent cough, dyspnea and bloody sputum and was subsequently admitted to our hospital. The following results were obtained from laboratory tests: leukocyte count, 9,990/μL and C-reactive protein (CRP) concentration, 14.57 mg/dL. On admission, thoracic X-ray and CT scans revealed oval lesions in the bilateral lungs, and a specimen was collected for a microbiological examination. The collected specimen was smeared, and both Gram staining and Kinyoun’s acid-fast staining were performed. The Gram staining revealed the cells to be Gram-positive, coccoïd, with thin branching filaments (Fig. 1). On Kinyoun’s acid-fast staining, the cells presented as thin, acid-fast, branching filaments. The specimen was inoculated on 5% sheep blood agar, incubated at 35°C, and...
examined daily for three days for the direct microscopic examinations.

The colony formed by the clinical isolate was white. In addition, the clinical isolate was found to be an aerobic, Gram-positive, rod-shaped bacterium approximately 1×7 μm in size, as identified according to standard bacteriological methods.

The antibacterial susceptibility of the *Nocardia* strain was determined using the microdilution method. The isolated organism was resistant to sulfamethoxazole/trimethoprim [minimum inhibitory concentration (MIC), >80 μg/mL], ceftriaxone (MIC, >4 μg/mL), minocycline (MIC, 2 μg/mL), clindamycin (MIC, ≥1 μg/mL), levofloxacin (MIC, ≥8 μg/mL), gatifloxacin (MIC, ≥4 μg/mL), and moxifloxacin (MIC, ≥4 μg/mL), and sensitive to imipenem (MIC, ≤0.06 μg/mL). The susceptibility to amikacin could not be determined at our hospital. The antimicrobial drug was changed to imipenem/cilastatin (1 g/day) and amikacin (200 mg/day) based on the above susceptibility findings.

The patient’s fever was alleviated on the sixth day of hospitalization. In addition, the knot and permeation shadows on chest CT images disappeared, and the degree of inflammation, as indicated on blood tests, improved. The medical treatment was continued for eight weeks.

Molecular identification was carried out via PCR amplification and a sequencing analysis. A 1,433-base pair (bp) sequence of the 16S rRNA gene was amplified with universal primers 8UA (5’-AGAGTTTGATCMTGGCTCAG-3’) and 1485B (5’-ACGGGCGGTGTGTRC-3’), and the purified PCR products were sequenced with an ABI Prism Big Dye sequencing kit (Applied Biosystems, Tokyo, Japan). The tree topology was evaluated using bootstrap analysis with the Clustal W software program (Applied Biosystems, Japan), and the isolate was identified to be *N. elegans* with 100% sequence identity to the type strain.

Based on the 16S rRNA gene sequence, we identified the isolate to be *N. elegans*, and the diagnosis was confirmed according to the DNA Data Bank of Japan (DDBJ), the sequence of the 16S rRNA gene was 100% identical to that of the *N. elegans* strain (Fig. 2).

A proteome analysis was attempted using MALDI-TOF MS. Three colonies grown on blood agar were centrifuged at 5,000 Xg at 4°C for five minutes, and the precipitate was placed on the pallet of the MALDI-TOF MS machine. After placind and drying 1μL of pellets on the HCCA-Matrix, we measured the MS. The MS was carried out in the Autoflex-Speed Linear mode, and the results were analyzed using the Biotyper software 3.0 program (Bruker Daltonics, Billerica, USA). The score for *Nocardia elegans* DSM was 1.76; therefore, the similarity was adequate to confirm the identification of *N. elegans*.

*Nocardia* is primarily an opportunistic infection. Symptoms most commonly develop in patients with autoimmune diseases and malignant tumors under treatment with steroids and immunosuppressants, as well as those with chronic lung diseases. There are many reports regarding the development of symptoms of nocardiosis in patients with other conditions, including chronic alcoholism, diabetes and AIDS. The major type of disease is pulmonary nocardiosis. The symptoms are non-specific, although coughing, purulent sputum production, respiratory pain, and a high fever are thought to be the major symptoms. Symptoms are subacute in many cases, with permeation and knot shadows of a non-zone nature and cavity formation observed on chest radiography and CT (9, 10). A clinical sample must be identified in order to confirm the diagnosis (11). The first choice of medical treatment is antibiotic therapy with sulfamethoxazole/trimethoprim, while second choices include imipenem, amikacin, minocycline, and ceftriaxone due to their side effects, such as liver damage (12). In severe cases, two or three antibiotics are used. In the present case, the patient was diagnosed clinically at the time of hospitalization. Moreover, because he was in an immune suppressed state owing to medical treatment for an AOSD, combined therapy with imipenem and amikacin was administered. As a result of the antibiotic therapy, his general state and laboratory findings improved.

*Nocardia* sp. is an environmental bacterium distributed in excrement, soil, water, and decaying plants and animals. The causative pathogen is impossible to identify using existing

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**Figure 1.** Gram staining revealed that the cells were Gram-positive, coccoid within branching filaments.

**Figure 2.** The sequence of the 16S rRNA gene was 100% identical to that of the *N. elegans* strain.
laboratory culture methods, and must be confirmed according to a gene analysis of the separated bacillus.

The identification of *Nocardia* sp. has always been considered a time-consuming task in routine laboratory work, and some publications have proposed tools to improve, accelerate, and ease this process. MALDI-TOF MS is a new method for performing microbiology laboratory work that is capable of confirming the identity of several microorganisms known to be difficult to identify using conventional tests. Verroken et al. established a method for rapidly and easily identifying *Nocardia* sp., including *N. elegans*, and reported the advantages of MALDI-TOF MS (13). Their method involves identifying a microorganism by searching a library for the ribosomal 16S protein. Furthermore, MALDI-TOF MS demonstrates high sensitivity for rapidly and easily identifying *Nocardia species* (13); however, the high cost of dedicated MALDI-TOF devices is comparable with that of laboratory equipment (13). Using the MALDI-TOF method, we were able to identify *N. elegans* as the causative pathogen in the present case. Six cases of Nocardiosis caused by *N. elegans* have been previously reported, including one case involving a cat (8); however, the current case is the first reported case in which 16S rRNA sequencing and the MALDI-TOF MS method were used to identify the clinically isolated pathogen. The MALDI-TOF MS method is useful for more rapidly identifying pathogenic microorganisms than the 16S rRNA sequencing method.

**The authors state that they have no Conflict of Interest (COI).**

**References**


