First Spanish Case of Nocardiosis Caused by *Nocardia takedensis*\(^v\)

A. Betrán,\(^1\) A. Rezusta,\(^1\) M. A. Lezcano,\(^1\) M. C. Villuendas,\(^1\) M. J. Revillo,\(^1\) P. Boirón,\(^2\) and V. Rodríguez-Nava\(^2\)*

Microbiología, Hospital Universitario Miguel Servet, Instituto Aragonés de Ciencias de la Salud (I+CS), Paseo Isabel la Católica 1-3, 50009 Zaragoza, Spain,\(^1\) and Université de Lyon, France, Research Group on Bacterial Opportunistic Pathogens and Environment, CNRS, Faculté de Pharmacie, Lyon 1, UMR 5557 Ecologie Microbienne, Observatoire Français des Nocardioses, 8 avenue Rockefeller, 69373 Lyon, France\(^2\)

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*Nocardia takedensis* is a recently described species isolated from soil. The first clinical isolate in Japan has recently been reported. This report describes the first clinical isolate of *N. takedensis* in Spain from a respiratory specimen.

*Nocardia* species are aerobic gram-positive bacteria of the Actinomycetales order, soil saprophytes involved in the decomposition of plant material (2, 7, 10). Isolation from respiratory samples may be indicative of colonization or invasive infection. The criteria used to determine the clinical significance of a culture positive for a *Nocardia* sp. include signs and symptoms in the patient and the clinical setting (e.g., corticosteroid therapy, transplantation). The respiratory tract is the most common site of infection or colonization. Most *Nocardia* infections are believed to be acquired by inhalation of airborne spores or mycelial fragments from environmental sources (3). The diagnosis of nocardiosis is usually based on direct examination since conventional cultures are complex, as well as long and time-consuming (4). However, the classical identification process is complicated and incomplete and therefore the current identification of *Nocardia* is being mainly based on molecular phylogenetic information (3). With the introduction of genetic technologies, the reports of new species of *Nocardia* have increased. *Nocardia takedensis* has been recently described as a new species of the genus (14). The type strain of this species was isolated from soil, and only a clinical case of a patient with T-cell lymphoma has been reported in Japan so far (13). This report describes the first clinical isolate of *N. takedensis* from a respiratory specimen in Spain.

The strain of *N. takedensis* was isolated from a 41-year-old female patient with type 2 diabetes mellitus, cosinophilic granuloma, and lung damage. The patient was diagnosed after a lung biopsy with typical histological findings. She was successfully treated with steroids for about 6 months.

In the laboratory, sputum was processed for mycobacterial study so the strain was isolated from mycobacterial culture medium. Gram-stained smears revealed gram-positive short filaments, cocccoid forms, and branching rods. In the modified Ziehl-Neelsen stain, the strain was partially acid fast. The identification to the genus level as *Nocardia* was based on macroscopic, microscopic, and biochemical characteristics.

The methods described by Boirón et al. (1) were used to determine the decomposition of adenine, casein, hypoxanthine, tyrosine, and xanthine. The isolated strain did not degrade any of them. Regarding the culture conditions, this strain grew well at 30°C but did not grow at 45°C.

DNA was extracted for PCR amplification and sequencing on both strands of the 16S rRNA gene as previously described by Rodriguez-Nava et al. (10). A 606-nucleotide amplified fragment was obtained with primers described by Rodriguez-Nava et al. (10) (Noc1, 5′-GCTTAACACATGCAAGTCG-3; Noc2, 5′-GAATTCCAGTCTCCCCTG-3), confirming the diagnosis of nocardiosis. The clinical isolate sequence was then compared with those of representatives species classified in the genus *Nocardia* in the GenBank and BIBI databases (5). Our clinical isolate was identified as *N. takedensis* with 99.5% sequence similarity (3 nucleotide differences out of 606 nucleotides) to the type strain of *N. takedensis*. It has been demonstrated by 16S rRNA gene sequencing that *N. takedensis* is most closely related to two pathogenic *Nocardia* species, *N. beijingensis* (98.1 to 98.3% similarity) and *N. brasiliensis* (97.9 to 98.0% similarity) (14), which have been reported as responsible for pulmonary abscesses and actinomycetoma, respectively.

The susceptibility of the isolate to different antimicrobials was determined by using a commercial broth microdilution method. Appropriate dilutions for MIC determinations were obtained from EMIZA 9EF Sensititre plates. Reference strains *Escherichia coli* ATCC 35218 and *Staphylococcus aureus* ATCC 29213 were used as controls (8). We used the recommended primary antimicrobials (aminocaricin, amoxicillin-clavulanic acid, ciprofloxacin, imipenem, tetracycline, and trimethoprim-sulfamethoxazole) and one secondary antimicrobial (cefotaxime) for susceptibility testing. The plate was incubated at 37°C for 72 h and read manually with a mirrored box.

*Nocardia* species can vary in their antimicrobial susceptibility patterns (11). In vitro, this isolate was susceptible to amikacin (≤4 μg/ml), cefotaxime (8 μg/ml), trimethoprim-sulfamethoxazole (2 and 38 μg/ml, respectively), imipenem (4 μg/ml), and tetracycline (≤4 μg/ml) and was resistant to amoxicillin-clavulanic acid (≥16/8 μg/ml) and ciprofloxacin (≥4 μg/ml). We used Clinical and Laboratory Standards Insti-
The patient did not show any symptoms of pulmonary nocardiosis or any radiographic manifestations, so this isolation represented transient colonization. An association such as the one seen in our patient between nocardiosis and steroid therapy has already been reported in previous studies (12). Pulmonary nocardiosis should be suspected in patients with a history of prior steroid use since this risk factor has been highly correlated with the development of nocardiosis (6, 9). The patient reported here was an immunocompromised patient who received steroid treatment, so it is possible that aerosolization of grass and soil facilitated the lung colonization seen.

In conclusion, the colony morphology or morphological characteristics of Nocardia do not allow differentiation of the numerous species. The new molecular methods based on the 16S rRNA gene for Nocardia identification are crucial. Besides, the different species of Nocardia show species-specific drug susceptibility patterns, and patients are most frequently immunosuppressed and generally require antibiotic treatment. Finally, reports of isolates from clinical specimens of new species such as N. takedensis underline the need to provide clinical data to establish their significance in every patient but especially in patients with risk factors.

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REFERENCES


