Isolation of *Nocardia beijingensis* from a pulmonary abscess reveals human immunodeficiency virus infection

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Abstract

A strain of *Nocardia* was isolated from a pulmonary abscess of a human immunodeficiency virus-infected patient in France. Comparative 16S rRNA gene sequence analysis revealed that the isolate represented a strain of *Nocardia beijingensis*. Antimicrobial-susceptibility testing was essential to guide the clinicians to successfully treat this infection.

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The strain of *Nocardia beijingensis* was isolated from a 47-year-old male who was admitted to our unit on 23 December 2010 for chest pain and a history of amoxicillin-resistant pneumonia. His main medical history was chronic sinusitis. On examination, he appeared asthenic, without fever. Physical examination concluded there was deteriorated general status, with a weight loss of 5 kg in 8 weeks. Laboratory test results were hemoglobin, 11.9g/dL; neutrophils, 13G/L; lymphocytes, 1.2G/L; platelets, 357G/L; and C-reactive protein level, 241mg/L (normal range <6). A chest radiographic disclosed mediastinal widening with a left para-aortic mass. A computed-tomography scan of the chest revealed an abscess (Figure 1).

Blood cultures were negative.

A bronchoalveolar lavage and a lung biopsy of the abscess were performed. In the laboratory, sputum was processed for mycobacterial study: the strain was also isolated from the mycobacterial culture medium. In a modified Ziehl–Neelsen stain, the strain was partially acid fast. The samples were tested for tuberculosis by PCR on the MTB/RIF test platform (GeneXpert, Cepheid) and were negative except for, interestingly, one of the five probes that target the *rpoB* gene. Gram-stained smears revealed Gram-positive short filaments, coccoid forms, and branching rods. Primary cultures from the pulmonary abscess and a bronchoalveolar lavage on blood- and chocolate-agar plates, incubated at 37°C in 5% CO₂, yielded, within 3 days, small, chalky white, dome-shaped rough colonies. On Coletos medium, the colonies appeared irregular and orange colored (Figure 2). They were Gram-positive rod-shaped organisms producing aerial hyphae. They were partially acid-fast, and were considered most likely to represent *Nocardia* spp.

Antibiotic susceptibility among isolates within the genus *Nocardia* is unpredictable (15); therefore, we assessed susceptibility according to the guidelines of the French Society for Microbiology, using the disk-diffusion method or the Etest (AB Biodisk, Solna, Sweden) on
Mueller–Hinton plates. Although broth microdilution is now recommended by the Clinical and Laboratory Standards Institute (CLSI), two reports suggest that MIC determination by broth microdilution and the Etest give similar results (1, 2), making the Etest suitable and more convenient for routine work. The results were recorded after 48h (or after 72h if growth was insufficient after 48h) and interpreted according to the MIC breakpoints published by the CLSI. However, the Etest method was not available for every antibiotic in our laboratory. Therefore, the agar disk-diffusion method on Mueller–Hinton plates, in ambient air, was used for the other antibiotics.

In the absence of consensual breakpoints, the interpretation of inhibition zones was made according to the proposed guidelines of the French Society for Microbiology. The profile showed resistance to penicillin (ampicillin MIC 90 > 128µg/mL) and vancomycin (MIC90 > 8µg/mL), susceptibility to imipenem (MIC90 = 0.032µg/mL), aminoglycosides, tetracyclines, tigecycline, linezolid (MIC90 = 0.064µg/mL), quinolones (ciprofloxacin MIC90 = 0.5µg/mL), cotrimoxazole, and 3rd-generation cephalosporins (cefotaxim and ceftriaxone MIC90 = 0.125 and 0.4µg/mL respectively), but intermediate susceptibility to meropenem and ertapenem. Prior to the isolation of the *Nocardia*, the patient was empirically treated by ampicillin. Once the diagnosis of nocardiosis was made (based on culture results), and before the availability of drug-susceptibility results, the treatment was switched to imipenem and amikacin. Finally, based on the susceptibility results, this treatment was continued for 3 weeks. This resulted in rapid clinical improvement and intravenous to oral shift therapy was practised with cotrimoxazole for a total duration of antibiotic course of 3 months. A complete resolution of pulmonary symptoms was observed. A cranial CT scan revealed no cerebral abscess. Moreover, regarding the usual association with immunosuppression, a HIV serology revealed seropositivity with a HIV viral load of 47,833 copies/mL, and a CD4+ lymphocyte count of 39/mm³; thus, specific treatment was initiated.
The strain was identified to the genus and species levels by 16S rRNA gene-targeted PCR. Briefly, suspensions were made of a single bacterial colony in 20µL of PCR-grade water, and the suspension was treated using an 800W microwave oven for 15s at 480W, as previously described (14). Cellular debris was pelleted at 12,000g for 10min, and the supernatant containing the genomic DNA was used in the PCR assay. DNA was amplified with primers for Forward (5-AGTTTGATCMTGGCTCAG-3) and Reverse (5-GGACTACHGAGGTATCTAAT-3), for 35 cycles in a LightCycler 2.0 (Roche Diagnostics, Meylan, France). Amplification reactions were performed in a volume of 20µL, which contained 4µL of DNA template, 4 mM MgCl₂, 0.25µM of each primer, and 2µL of 10x LightCycler FastStart DNA Master SYBR Green I mixture (Roche Diagnostics, Meylan, France).

Following an initial denaturation at 95°C for 10min, and 35 cycles of denaturation at 95°C for 10s, annealing was done at 54°C for 5s, and extension at 72°C for 40s. The amplicons were then sequenced by a PCR-based reaction using the Big Dye Terminator (Applied Biosystems Inc) method, according to the manufacturer’s instructions, and were detected in an AbiPrism 3500xL automatic DNA sequencer (Applied Biosystems Inc.). The 500-bp amplified DNA sequence was 100% homologous with that of Nocardia beijingensis (GenBank sequence accession number AB094648; NCBI BLAST and BiBi [http://pbil.univ-lyon1.fr/] phylogenetic tools). Because of the unusual isolation of this strain, confirmation was performed by the “Observatoire Français des Nocardioses” (Pr Boiron, Lyon, France), based on chemotaxonomical characteristics plus sequence analysis of 16S rRNA.

Nocardiosis are infections caused by soil-borne aerobic microorganisms belonging to the genus Nocardia. Over 90 species of Nocardia have been described (http://www.bacterio.cict.fr/n/nocardia.html), and several are recognized as human and/or animal pathogens, causing diseases ranging from pulmonary or central nervous-system...
infections in immunocompromised patients (~60% of all reported cases of nocardiosis are associated with underlying immunosuppression (13)) to cutaneous infections in normal hosts (4). In France, on the basis of the numbers of Nocardia strains referred to the National Reference Center for Mycoses and Antifungal Agents, Institut Pasteur, Paris, in the period from 1987 to 1990, it was estimated that between 150 and 250 cases of nocardiosis are diagnosed in France each year (3). The species previously know as Nocardia asteroides complex was the most commonly identified species and accounts for 71.4% of pulmonary infections, 80.0% of central nervous-system infections, and 80.0% of systemic infections (14), with the rest being caused by N. farcinica and N. nova. N. asteroides complex has been redistributed among several species and N. asteroides sensu strictu is now considered as an uncommon cause of infections (4, 7).

In HIV-positive patients, Nocardia species are well-known agents of opportunistic infections. The frequency of Nocardia infection in HIV-infected patients has increased during the past few years and, although it is not of great concern as an AIDS-associated infection, its nonspecific clinical presentation in these patients and the slow growth of the bacilli may be confused with other lung infections, such as tuberculosis (11). The mortality rate can be high at 40–60% (11, 12), and successful treatment depends on early recognition of the nocardial infection and the initiation of a specific treatment. In 2001, a new species of Nocardia, N. beijingensis, was described by Wang et al., isolated from a soil sample from a sewage ditch at Xishan mountain in Beijing (17). Strains have been isolated in Asia between 2004–2010 from patients with nocardiosis (5, 9, 10, 15, 16), mainly from pulmonary samples (80%) and in immunocompromised patients. To our knowledge, this is the first infectious case reported outside of Asia, although some strains have been isolated in Europe, but without strong evidence of their involvement in pathology (6). Besides, our patient never went to these countries.
If morphological and biochemical tests are unable to discriminate the species, it has been proposed that *N. beijingensis*, *N. farcinica*, and *N. brasiliensis* may be differentiated by their drug-susceptibility pattern, but certain differentiation relies on molecular methods. *N. beijingensis* is susceptible to imipenem, tobramycin, and kanamycin; *N. brasiliensis* is susceptible to tobramycin, but resistant to imipenem and kanamycin; and *N. farcinica* is susceptible to imipenem, but resistant to tobramycin and kanamycin (10).

In conclusion, the colony morphology or morphological characteristics of *Nocardia* do not allow differentiation of the numerous species. The molecular methods based on the 16S rRNA gene for *Nocardia* identification are crucial. Also, the different species of *Nocardia* show species-specific drug susceptibility patterns, and patients are most frequently immunosuppressed and generally require antibiotic treatment. Susceptibility testing of all isolates may be imperative as more variability in susceptibilities is being recognized in isolates and new species (8). Therefore, the treatment of choice for *Nocardia* infections is cotrimoxazole; however, the bacteria are usually also susceptible to imipenem, amikacin, minocycline, and 3rd-generation cephalosporins. Our patient recovered with prolonged cotrimoxazole treatment after an initial course of imipenem and amikacin. The present report highlights the putative role of *N. beijingensis* as an agent of pulmonary-abscess infection in a HIV-positive patients. In addition to the unusual causative species, nocardiosis is a rare primary feature of a HIV seropositivity. Finally, reports of isolates from clinical specimens of new species, such as *N. beijingensis*, underline the need to provide clinical data to establish their relevance in every patient, but especially in patients with risk factors.
References


Figure 1: CT scan of the patient’s chest showing a left para-aortic mass of ~7 cm (white star), within the main area, with a necrotic centre (mediastinal window).

Figure 2: Shape and colour of colonies on Coletaos (A) and chocolate (B and C) media.