First Case of Cerebral Abscess Due to a Novel Nocardia Species in an Immunocompromised Patient

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We report the first case of cerebral abscess due to a novel species of Nocardia in a heart transplant patient and describe the antimicrobial susceptibility of this isolate. As our patient was intolerant to trimethoprim-sulfamethoxazole, we also discuss alternative therapeutic options in brain abscess due to Nocardia sp.

CASE REPORT

A 37-year-old woman underwent heart transplantation in November 2007 in the University Hospital of Lille (France). A rejection occurred in April 2008 (grade IR on International Society for Heart and Lung Transplantation classification), requiring an increase of the corticosteroid therapy. She was maintained on cyclosporine (150 mg twice a day [b.i.d.]), mycophenolate mofetil (500 mg/day), and prednisone (10 mg/day). Trimethoprim-sulfamethoxazole (TMP/SMX) (80/400 mg/day) prophylaxis for Pneumocystis jirovecii was discontinued in March 2008, according to our Heart Transplant Center’s protocol. In June 2009, after a pneumonia was treated with ceftazidime for 2 weeks, the computed-tomography (CT) scan revealed multiple bilateral nodules, one of them cavitated, and bilateral pulmonary infiltrate with hilar adenopathies. The cultures from the bronchoalveolar lavage (a few days after ceftazidime discontinuation) were sterile, and the biopsy specimens showed nonspecific inflammation. In August 2009, she presented with fever and seizures. The leukocyte count was 5,950/mm³, and C-reactive protein was measured at 6 mg/liter. The cerebral CT scan and magnetic resonance imaging (MRI) showed a left frontal brain abscess with perilesional edema. The direct Gram stain of the abscess liquid was negative, but cultures recovered colonies consistent with Nocardia with Gram-positive filaments. The isolate grew on Columbia agar plates as white, rough, irregular, dry, 2-mm-diameter colonies after 48 h of incubation at 37°C in an aerobic atmosphere. Blood cultures and cerebrospinal fluid remained sterile.

The antimicrobial susceptibility of this strain (OFN 09.174) was tested by using a broth microdilution method according to the CLSI standard M24-A2 guidelines (1). The MICs were determined with primers SQ1 (5’-AGAAGTATGCTMTGCCATG-3’) and SQ6 (5’-CCGTTGTCAGACGCCC-3’) (3). For gyrB, a fragment of about 1,230 nt was also amplified by using primers GYRB01 (5’-ATGGCCTTCTCTCAACAGGG-3’) and GYRB02 (5’-GGTTCAGCTGCATCGGATCT-3’) and sequenced according to a method described by Shen et al. (4).

As shown below, four genes (16S rRNA, gyrB, hsp65, and sod) have been used in the genetic analysis with the aim of identifying our isolate at the species level. However, the multigenic analysis was not enough to correctly identify the isolate, so a DNA-DNA hybridization with the most closely phylogenetically related Nocardia species was necessary to conclude the identification process. Strain OFN 09.174 was grown in Bennett medium, and no-cardial DNA extraction (2) was performed. For 16S rRNA, the nearly complete gene sequence (1,315-nucleotide [nt] fragment) was determined with primers SQ1 (5’-AGAAGTATGCTMTGCCATG-3’) and SQ6 (5’-CCGTTGTCAGACGCCC-3’) (3). For gyrB, a fragment of about 1,230 nt was also amplified by using primers GYRB01 (5’-ATGGCCTTCTCTCAACAGGG-3’) and GYRB02 (5’-GGTTCAGCTGCATCGGATCT-3’) and sequenced according to a method described by Shen et al. (4). For the hsp65 gene, a fragment of 441 nt was amplified by using TB01 (5’-ACC AACGATGGTGTGTTCCAT-3’) and TB02 (5’-CTTGTCAACC GCATACTCT-3’) primers (2). Finally, for the sod gene, a fragment of 442 nt was amplified by using Z205 (5’-ACGTTCACCA CGGCGCAGCA-3’) and Z212 (5’-TGGGCGCCAGTTCAAGA GTT-3’) primers (5). PCR products were purified and sequenced on both strands. The resulting sequences were aligned.

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with the corresponding sequences of representative Nocardiopsis species obtained from GenBank by using Clustal X software (6).

The phylogenetic study was performed using MEGA 5 software (7). We obtained evolutionary trees with our strain and the type strains of the most closely phylogenetically related species. These evolutionary trees were inferred with three treeing algorithms: the maximum-likelihood (8), maximum-parsimony (9), and neighbor-joining (10) methods. The robustness of the trees was tested by bootstrap resampling (1,000 replicates each).

The PCR product for the 16S rRNA gene showed a sequence similarity of 99.5% to N. beijingensis JCM 10666T, 99.4% to N. araoensis DSM 44729T, 99.1% to N. arthritidis DSM 44731T, 99.0% to N. amikacinitolerans DSM 45339T, 98.9% to N. niwae DSM 45340T, and 98.7% to N. asiatica DSM 44668T. As shown in the phylogenetic tree of 16S rRNA (Fig. 1), strain OFN 09.174 forms a clade with these strains. The phylogenetic tree shown in Fig. 1 shows that strain OFN 09.174 forms a clade with the type strains of the most closely related Nocardiopsis species.

With the hsp65 gene, we obtained a PCR product sequence with a sequence similarity of 98.4% to N. asiatica DSM 44668\textsuperscript{T}, 98.2% to N. araoensis DSM 44729\textsuperscript{T}, 97.6% to N. niwae DSM 45340\textsuperscript{T}, 97.3% to N. abscessus DSM 44432\textsuperscript{T}, and 97.3% to N. pneumoniae JCM 12119\textsuperscript{T}. No data are available regarding N. amikacinitolerans DSM 45339\textsuperscript{T}. The phylogenetic tree shown in Fig. 1 shows that strain OFN 09.174 forms a clade with these strains.

Finally, using the sod gene, the PCR product showed a sequence similarity of 98.0% to N. niwae DSM 45340\textsuperscript{T}, 97.3% to N. abscessus DSM 44432\textsuperscript{T}, 97.0% to N. beijingensis JCM 10666\textsuperscript{T}, 97.0% to N. gamkensis DSM 44956\textsuperscript{T}, 96.8% to N. arthritidis DSM 44731\textsuperscript{T}, and 96.6% to N. asiatica DSM 44668\textsuperscript{T}. No data are available regarding N. amikacinitolerans DSM 45339\textsuperscript{T}. The phylogenetic tree in Fig. 1 shows that strain OFN 09.174 forms a clade with all the strains listed before.

DNA-DNA hybridization studies were performed using the DNAs of strain OFN 09.174 and the most closely phylogenetically related Nocardia species previously obtained in phylogenetic studies. Two criteria were used to choose these species: the number of times that a species is present in the same clad as our strain and the similarity between those strains. We chose N. niwae and N. asiatica, which appear in the same phylogenetic cluster as strain OFN 09.174 regardless of the gene under study, and N. beijingensis and N. arthritidis, which appear in the same phylogenetic cluster as our strain for three genes. Finally, of the species that appeared in the same cluster of our strain for only two genes, we chose those that presented the higher level of similarity, namely, N. araoensis and N. abscessus. Therefore, N. gamkensis, N. amamiensis, and N. pneumoniae were discarded. Regarding N. amikacinitolerans, even though no data for hsp65 and sod genes of this species are available in databases, the similarity level determined using the gyrB gene was so low that we were also able to discard this species for further studies.

Strain OFN 09.174 showed a DNA-DNA relatedness of lower than 70% with all the species listed above.

We can conclude that the OFN 09.174 isolate represents a new species because previous results do not allow us to identify our isolate as being an already-described species. We propose to name this new species “Nocardia lillensis” (referring to Lille, where the strain was isolated).

Further studies, including investigation of morphological, physiological, and biochemical characteristics and analysis of cell composition, will be done for completing the characterization of this isolate, which will allow us to publish later the description of the new species to which the N. lillensis OFN 09.174 strain belongs.

**Discussion.** The risk factors (heart transplantation, immunosuppressive treatment that includes administration of corticosteroids) as well as the clinical presentation were classic for nocardiosis in our patient. The pneumonia observed 2 months before the diagnosis was possibly already a pulmonary nocardiosis. An initial treatment with ceftriaxone might have been responsible for the negative bronchoalveolar lavage fluid cultures, although the absence of associated pulmonary nocardiosis has been described in up to 34% of patients with nocardial brain abscesses.

The initial empirical treatment with TMP/SMX and cefotaxime was appropriate to the antimicrobial susceptibility of our strain. After TMP/SMX was discontinued due to the development of neutropenia, the patient was treated with ciprofloxacin and cefotaxime. Ciprofloxacin was chosen as a companion drug, despite a MIC at 4 µg/ml (at the cutoff between intermediate susceptibility and resistance), for its tolerance profile and excellent diffusion in the central nervous system (CNS). We expected that continuing high-dose treatment might be beneficial, although not optimal. We did not consider it reasonable to introduce linezolid, in this context of drug-related acute neutropenia. After 6 weeks, we considered a switch to an oral treatment, for a total
duration of 1 year, and decided to introduce minocycline in association with ciprofloxacin.

Linezolid might have been a better option; indeed, there is increasing evidence for its efficacy in disseminated nocardiosis, including cerebral abscesses, with long-lasting treatments (2 to 24 months) (11–16). However, anemia and thrombocytopenia are frequent with prolonged treatments (12, 14, 15), and rare but severe adverse events such as lactic acidosis, neuropathy, and vi-
Nocardia farcinica was not possible to use it in our patient because of gastrointestinal
use the technique of multilocus sequence typing (MLST) with at
gene has its limitations due to the lack of polymorphism of this
sequencing of the complete 16S rRNA gene as the sole reference
species that have made some clusters bigger or have even led to the
Nocardia characterization. Actually, in recent years, the taxonomy of
The use of minocycline as a second-line oral drug for CNS nocar-
ially severe side effects. Minocycline might be used when the
choice of a second-line oral therapy in patients with TMP/SMX
in association with trimethoprim-sulfamethoxazole for treatment
of cutaneous nocardiosis (18, 19). It was also efficient as a main-
tenance treatment after an initial 3 to 5 weeks of intravenous treat-
ment in pulmonary nocardiosis (20, 21). Regarding nocardial
CNS infection, Wren et al. reported a successful treatment with
minocycline at 400 mg/day. The isolate was highly susceptible (MIC = 0.049 μg/ml), and drug concentrations obtained in the
CNS were 16 to 22 times higher than the MIC (22). Leitersdorf et
al. reported an uneventful recovery in 9 out of 10 transplanted
patients treated with minocycline (100 to 600 mg/day) for dissemi-
nated nocardiosis, including CNS infections (23). Two more
cases of successful maintenance treatment with minocycline at
100 mg/day in patients with nocardial cerebral abscesses have also been reported (11, 24). However, a treatment failure has been
described in three patients treated with minocycline for pulmo-
nary nocardiosis, with the patients developing brain abscesses un-
der treatment. Susceptibility testing was not available in two cases,
but the strain was susceptible in vitro in the third patient (15, 25).
The use of minocycline as a second–line oral drug for CNS nocard-
dioidos should be limited to patients with a susceptible isolate
(MIC < 0.5 μg/ml) and requires a close follow-up of efficacy. It was not possible to use it in our patient because of gastrointestinal
side effects.

This study also showed the difficulties of Nocardia species characterization. Actually, in recent years, the taxonomy of
Nocardia has become more complex due to the description of new
species that have made some clusters bigger or have even led to the
creation of new ones. The use, with identification purposes, of
the sequencing of the complete 16S rRNA gene as the sole reference
gene has its limitations due to the lack of polymorphism of this
gene, especially when the strain under study belongs to a cluster
that hosts a high number of different species with a similarity level
higher than the “cutoff” determined by the CLSI for this gene.

It is for this reason that, in these cases, it may be necessary to use the technique of multilocus sequence typing (MLST) with at
least four genes (among the 16S rRNA, hsp65, gyrB, sod, rpoB, and secA1 genes). In our study, the results obtained with four genes
were not discriminant enough to identify the strain but at least led
us to determine a group of the most closely related type species.
When the use of several genes is not discriminant enough, analysis
of DNA-DNA hybridization between the strain under study and this
group of type species must be performed in order to deter-
mind exactly the species the strain belongs to or, as happened in
this study, to conclude that the strain belongs to a new species.

In conclusion, this report describes a disseminated infection with CNS involvement by a new Nocardia species identified by a
molecular method. Despite a wide range of available drugs, the
choice of a second-line oral therapy in patients with TMP/SMX
intolerance is limited. Linezolid is highly efficient, but its potential
as a maintenance treatment is limited, due to frequent and poten-
tially severe side effects. Minocycline might be used when the
strain is fully susceptible, with a close follow-up of efficacy analy-
sis, in the absence of other therapeutic options. In severe cases, a
long course of intravenous antibiotic administration might be re-
quired.

Nucleotide sequence accession numbers. The nucleotide se-
quences of the 16S rRNA, gyrB, hsp65, and sod genes from our
isolate were deposited in GenBank under the following accession
numbers: JX421754 (16S rRNA), JX437683 (gyrB), JX464217
(hsp65), and JX464218 (sod).

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