First case of disseminated human infection with *Nocardia cerradoensis*

Caroline Piau\(^a,b,c\)#, Mallorie Kerjouan\(^a,d\), Marc Le Mouel\(^a\), Solene Patrat-Delon\(^a,e\), Pierre-Louis Henaux\(^a,f\), Vanessa Brun\(^a,g\), Marie-Pascale Morin\(^a,h\), Philippe Gautier\(^a\), Veronica Rodriguez-Nava\(^i\), Samer Kayal\(^a,b,c\)\#.  

University Rennes-1, Medical School, Rennes, France\(^b\); IGDR-UMR 6290-CNRS, Université Rennes 1, Rennes, France\(^b\); CHU Rennes-Hôpital Pontchaillou, departments of Microbiology\(^c\), Pulmonology\(^d\), Infectious Diseases\(^e\), Neusurgery\(^f\), Radiology\(^f\), Nephrology\(^h\), Rennes France; Observatoire Français des Nocardioses UMR 5557-CNRS, University Lyon 1, Lyon, France\(^i\).  

Running Head: Disseminated *Nocardia* infection

# Address correspondence to Samer KAYAL, samer.kayal@chu-rennes.fr or Caroline PIAU, caroline.piau@chu-rennes.fr.
Herein we report the first human disseminated infection with *Nocardia cerradoensis* in a renal transplant patient, and isolated after a brain biopsy. Species identification was based on 16S rRNA, *gyrB* and *hsp65* gene analyses. Antibiotic treatment was successful by combining carbapenems and aminoglycosides, before switching to oral trimethoprim-sulfamethoxazole.
A 59 year-old woman, with a history of end-stage renal disease, secondary to autosomal–dominant polycystic kidney disease, received a renal transplant in 2010. Immunosuppressive regimen included tacrolimus 12 mg/day and prednisolone 7.5 mg/day. Prophylaxis with trimethoprim-sulfamethoxazole (SXT) was given for six months after the transplantation and then stopped due to intolerance symptoms. She had no history of recent travel. Two weeks before admission she had had a mild headache with fever, and chills and she was empirically treated with amoxicillin and prednisone for seven days. After a brief improvement, she developed a non-productive cough with dyspnea. Based on the hypothesis of pneumonia, antibiotic treatment was switched to ceftriaxone combined with aerosol bronchodilators. Pulmonary symptoms rapidly worsened, and on September 2013 she was admitted to hospital for further investigations.

On examination, the patient was awake and complained of inspiratory dyspnea. Diarrhea was noted a few days after the initiation of antibiotic treatment and resolved spontaneously. Temperature was measured at 37.8 °C, blood pressure was 150/90 mmHg, the pulse 97 beats/min, the respiratory rate 36/min, and oxygen saturation at 97% while she was breathing ambient air. The auscultation evidenced bilateral basal crepitus and no other abnormal sounds. Heart sounds were normal, abdomen was soft, without tenderness, distention, or organomegaly, and neurologic examination was normal. No peripheral lymph nodes were detected. Subcutaneous nodules of lower extremities appeared few days after she was admitted (Fig 1A). The white-cell count was 18.6 x 10^9/L, with 95% neutrophils and the blood level of C-reactive protein was 159 mg/L. Chest X-ray and computed tomography (CT) scan evidenced wall thickening of the right main bronchus, moderate ground glass opacities of the right upper lobe, a right hilar lymph node, a small right parenchymal nodule
(<10 mm), and a moderate homolateral pleural effusion (Fig1.B). Abdominal CT scan revealed diffuse peritoneal and right retroperitoneal nodules (Fig1.C), and a thickening of the cæcal wall. Bronchoalveolar lavage (BAL), bronchial and subcutaneous biopsies were performed and the examination of stained smears (Gram, Ziehl–Neelsen, May Grunwald Giemsa, Papanicolaou, Perls and hematoxilin safranin) showed no bacteria or abnormal cells.

Cultures for bacteria, fungi, and mycobacteria were all negative. Histological examination of the subcutaneous nodule concluded in an erythema nodosum. The amplification of bacterial 16S rRNA gene on the cutaneous biopsy was also negative. The diagnosis of primary lung carcinoma was then suggested and transbronchial right hilar lymph node puncture was performed and did not show any malignant cell; bacterial cultures were not done due to insufficient sampling.

On 20th day of hospitalization, the patient presented neurological worsening with generalized weakness, dysarthria, lateral seizures and pyramidal syndrome on examination. Brain CT-scan, without the administration of contrast material, followed by magnetic resonance imaging (MRI) showed no hemorrhage of brain parenchyma and small non-specific lesions suggesting a differential diagnosis between multiple abscesses and cystic/necrotic brain tumors (Fig 1.D). Stereotactic biopsy of brain parenchymal lesion was done, and Gram staining evidenced filamentous Gram-positive bacilli (Fig 1.E). Bacterial growth was obtained on chocolate and Buffered charcoal yeast extract plates (oriented by Gram staining) incubated at 37°C for 48 hrs in an aerobic atmosphere; the colonies of 2 mm diameter were white and rough. Forehead skin swab performed during neurological surgery and two positive aerobic blood culture (Bactec®), both plated on chocolate agar, allowed after 48 h of incubation the isolation of a bacteria with the same morphological features than above. Cerebrospinal fluid remained sterile after 10 days incubation.
Bacterial identification by Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF; Brucker®-France), even after a full extraction, yielded an identification of *Nocardia* species with a score value <1.7 (in 2014, 5627 species were included in the Brucker’s spectrometry database). To better identify all the isolated bacteria, we carried out the sequencing of *16S rRNA*, *gyrB*, and *hsp65* genes (1), which were queried against GenBank database. The best nucleotide identities (%) are respectively given for each gene: 1-*16S rRNA* gene: 99.84% with *N. cerradoensis* (2 different nucleotides [nt]/1325 nt fragment), 99.62% with *N. mikamii* (5/1325), 99.47% with *N. africana* and *N. aobensis* (7/1325) and 99.39% with *N. veterana* (8/1325); 2-*gyrB* gene: 98.98% with *N. cerradoensis* (10/990) and 98.28% with *N. mikamii* (17/990), 3-*hsp65* gene: 99.76% with *N. cerradoensis* (1/434) and 98.84% with *N. nova* *N. africana* and *N. aobensis* (5/434). Finally, the retained identification of isolated strain from brain abscess hereafter named OFN13.186 (Observatoire Français des Nocardioses; Lyon, France), is *N. cerradoensis*.

The antimicrobial susceptibility of the strain OFN13.186, was tested by using a broth micro-dilution method according to the CLSI standard M24-A2 guidelines (2). The MICs were studied as previously described (1), by using Rapid Growing Mycobacteria Plate Format (RAPMYCO) sensititre plates, incubated at 37°C for 72 h. Based on the previously established breakpoints for the *Nocardia* genus (1), the strain was resistant to amoxicillin-clavulanic acid, piperacillin-tazobactam, tobramycin, minocyclin and fluoroquinolones (Table 1). Taking into account the intolerance of the patient to SXT, the empirical intravenous treatment was started by associating meropenem (4 weeks) and amikacin (2 weeks), and allowed a rapid clinical improvement and a regression of all the neurological disorders. The patient was discharged, and after desensitization (3), antimicrobial treatment was switched to oral SXT for 6 weeks. Two months after the initiation of antimicrobial therapy, intra-abdominal...
nodules and thoracic lymph nodes had completely regressed, but a lung parenchymal nodule (5 mm) persisted. A follow-up of the pulmonary nodule, and a long-term prophylaxis with SXT (800/160) was then maintained.

The genus *Nocardia* is a ubiquitous group of environmental bacteria found in soil, water or decaying organic matter and more than 50 species have been already described (4, 5). Opportunistic infections to *Nocardia* in humans mostly occur in patients with cell-mediated immunodeficiency like transplant recipients. Herein, and as far we know, we report the first human case of diffuse nocardiosis due to the species *Nocardia cerradoensis* which has been recently described as a new species from a strain isolated in Cerrado soil in Brazil in 1985 as YT9 strain (6).

Immunosuppressive drugs required after kidney transplant induce immune cell deficiency formally recognized as a risk factor for diffuse nocardiosis (7). However nocardiosis remain a diagnostic challenge, because it is a rare etiology beside other opportunistic pathogens, clinical presentation is non-specific and the bacterial cultures are often difficult. The most common route of entry of *Nocardia spp* is inhalation or aspiration of the organism as they may become airborne, particularly on dust particles (4, 5, 8). In this case, the initial foremost respiratory symptoms and the main involvement of the right upper lobe and hilar lymph node, suggested that the respiratory tract is the primary site of infection. Although, primary cutaneous infection, or more occasionally spreading from oral cavity or gastrointestinal tract may occur, we believe the cutaneous nodules, that appeared after the onset of symptoms (Fig 1), as well as ceacal thickening and abdominal nodules, are associated with disseminated disease. Additionally, cutaneous nodules mimicking erythema...
nodusum or cellulitis have been previously described in disseminated infections (9). Unlike other bacterial brain abscesses, neurological symptoms in nocardiosis are highly variable, generally insidious, without fever or signs of septicemia. The most common signs are focal neurological deficits, non-focal findings, and seizures (10). In our patient, the acute CNS symptoms associated to multiple small cerebral abscesses were concomitant to a rapid progression of the dissemination since all the bacterial cultures became positive. However, the most likely is that the brain metastases were initially present despite a normal neurological examination.

At the onset of the infection, short antibacterial treatment (amoxicillin followed by ceftriaxone for two weeks) may explain the brief improvement of clinical status, and negative bacterial cultures despite the efforts to search a nocardiosis. *Nocardi* species exhibit a species-predictable antimicrobial susceptibility pattern and early species identification may be a crucial step to start an adapted antibiotic therapy (11). When bacterial culture were positive, the isolates were correctly identified with MALDI-TOF at the genus level and the lack of accuracy to identify at species level is easily explained because *N. cerradoensis* was not included in the database. Thus the identification of *N. cerradoensis* was obtained by the sequencing of three housekeeping genes (16SrRNA, *gyrB* and *hsp65*) as previously described (12, 13). The used criteria was actually, the one described by the MM18-A document i.e. for identification at species level whatever the used gene, and identity higher than 99,6% was required (14).

The probabilistic treatments in addition to their activity against *Nocardia* species should also exhibit good diffusion to all sites of infection. In our case, we decided to initiate the treatment with large spectrum antibiotics that also crosses blood-brain barrier. Actually, Linezolid, SXT and amikacin show the less percentage of resistance (11, 15). Carbapenem...
display most of the time low MIC and meropenem exhibit the best cerebral diffusion among this class. Time-kill studies showed that imipenem/amikacin and imipenem/moxifloxacin combinations were bactericidal for most isolates whereas linezolid and SXT exhibited mainly bacteriostatic activity (16). The noticed intolerance to SXT, and the initial identification limited to the genus level, did not allow us to make a decision for antibiotic treatment based on the in vitro predictable susceptibility. Therefore, the probabilistic treatment combined meropenem plus amikacin, for which the bacteria have subsequently been shown to be susceptible (Table 1). AST results, and patient improvement, lead us to reduce antibiotic spectra and start a treatment with SXT 800/160 after desensitization protocol (3). Prophylactic treatment was then instituted with SXT.

This is the first case of human disseminated infection with N. cerradoensis occurring in an immunosuppressed host. Nevertheless, even if a nocardiosis was initially strongly suggested among other opportunistic pathogens, it is essential to make the precise diagnosis at species level to institute the adapted antimicrobial regimen. Finally, because the Nocardia species can invade the CNS silently (17), we would like to emphasize, as recommended by others (18), that MRI of the brain should be considered systematically for the diagnostic evaluation even in the absence of neurological symptoms.

NUCLEOTIDE SEQUENCE ACCESSION NUMBERS

The GenBank accession numbers for the indicated genes nucleotide sequences of Nocardia cerradoensis (strain OFN 13.186) : gyrB gene (KP013615); 16S rRNA gene (KP013616); hsp65 gene (KP013617).
ACKNOWLEDGMENTS

This work was supported by the University Rennes1- Medical School, and CHU de Rennes-France. We would like to thank E. Bergeron and D. Mouniée for their technical support in this study.
REFERENCES


FIGURES

Figure 1

A

B

C

D

E
Figure 1: Disseminated nocardiosis. A- Subcutaneous nodules of lower extremities; B- Right hilar lymph node (*); C-Retroperitoneal nodules (arrows); D- Multiple Brain abscess (MRI); E- Gram stain (x100) of cerebral biopsy.
Table 1:

Legend for Table 1: Antimicrobial susceptibility testing results for the strain OFN13.186. MICs were determinate by broth microdilution method [see reference (1) for breakpoints and methods]. SXT= Trimethoprim-sulfamethoxazole.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Observed MICs</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>≤4</td>
<td>S</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>4</td>
<td>S*</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>&gt;32/16</td>
<td>R</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>≤4</td>
<td>S</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>≤4</td>
<td>S</td>
</tr>
<tr>
<td>Cefepime</td>
<td>≤4</td>
<td>S</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;4</td>
<td>R</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>≤2</td>
<td>S</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤2</td>
<td>S</td>
</tr>
<tr>
<td>Linezolid</td>
<td>≤4</td>
<td>S</td>
</tr>
<tr>
<td>Minocycline</td>
<td>2</td>
<td>I</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>2</td>
<td>I</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>16</td>
<td>R</td>
</tr>
<tr>
<td>SXT</td>
<td>≤1/19</td>
<td>S</td>
</tr>
</tbody>
</table>
Interpretative criteria used for amoxicillin was based on amoxicillin-clavulanic acid 252
break points (CLSI M24)