Report of an immunocompetent case with disseminated infection due to *Nocardia otitidiscaviarum*: Identification by 16S rRNA gene sequencing

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**SUMMARY**

*Nocardia otitidiscaviarum* belongs to the agents of opportunistic infections seen in immunocompromised patients, but may occur rarely in immunocompetent patients. In this report we described a case of a previously healthy 69-year-old woman with cerebral and retroperitoneal abscess due to *Nocardia otitidiscaviarum*. The patient was admitted to hospital because of loss of strength in her right arm and leg. *Nocardia spp.* was isolated from the abscess material. The intracranial lesions were drained by stereotactic craniotomy. The large abscess located around the left kidney was drained and microscopic examination of aspirated material showed *Nocardia spp.* For species identification, 16S rRNA gene sequencing was carried out and was 100% concordant with *Nocardia otitidiscaviarum*. Use of 16S rDNA gene sequencing for identification permits detection of rare aetiologic agents that cause brain abscesses.

**Keywords**: *Nocardia otitidiscaviarum*, disseminated nocardiosis, brain abscess, retroperitoneal abscess, 16S rRNA gene sequencing.

**INTRODUCTION**

Nocardiosis is an opportunistic infection caused by *Nocardia* spp. which may present with local (cutaneous, pulmonary) involvement and may also be generalized with the involvement of several organs. Nocardiosis has been reported more frequently in patients with deficient cell-mediated immunity such as found in AIDS, solid organ or haematopoietic stem cell transplant, chronic obstructive pulmonary disease, malignancies and cirrhosis. Long term steroid therapy has also been found to be associated with nocardiosis [1, 2].

Most human infections are caused by *Nocardia asteroides* complex which consists of six species including *Nocardia abscessus*, *Nocardia brevicatenana-paucivorans* complex, *Nocardia nova* complex, *Nocardia transvaalensis* complex, *Nocardia farcinica*, and *Nocardia asteroides*. Non-cutaneous invasive forms and central nervous system (CNS) involvement of the disease are typically related to this complex. Infections due to *Nocardia otitidiscaviarum* are uncommon (3%) when compared to those caused by other *Nocardia* species. This low incidence is associated with the reduced pathogenicity of *N.otitidiscaviarum* [3]. The most common presentation by *N.otitidiscaviarum* is pulmonary infection [4]. Here, we present a case with
intracranial and retroperitoneal abscess caused by *N. otitidiscaviarum* in an immunocompetent patient.

### CASE REPORT

A 69-year-old female presented with loss of strength in her right arm and leg for 10 days. Her medical history was unremarkable; she lived in a rural area and she had two dogs. On physical examination, the findings were as follows: temperature was 38.7 °C, blood pressure was 110/70 mmHg, pulse rate was 84/min. Respiratory and abdominal examinations were normal. Neurological examination of the patient revealed 3/5 strength in her right arm and leg. The results of laboratory investigations were as follows: white blood cell count was 12,0x10³ /µL with 68% neutrophilic leucocytes, CRP was 138 mg/L, sedimentation was 33mm/h. All the blood cultures were negative. Tests for HIV, hepatitis C virus, hepatitis B surface and core antigens were negative. The CD4 lymphocyte count was 263/mm³. Magnetic resonance imaging (MRI) of the brain showed bilateral multiple hemispheric lesions: the largest with a diameter of 35 mm x 2.5 mm located in right parietal lobe with an intense edema (Figure 1). Echocardiography and thoracic computed tomography were unremarkable. The patient was treated empirically with ceftriaxone 2g/day and metronidazole 2g/day. The lesions were sampled under the guidance of neuronavigation.

Microscopic analysis of the abscess materials yielded numerous polymorph nuclear leukocytes and filamentous-branched gram positive rods which stained positive with modified acid-fast stain (Figure 2). Abdominal ultrasound revealed a large lesion (130x90x80 mm) which was located around the left kidney. The lesion was drained and microscopic examination of the aspirated...
material was also compatible with *Nocardia* spp. So the diagnosis was “disseminated Nocardiosis” the empiric antibiotic therapy was changed to meropenem 6g/day and amikacin 1g/day.

**Microbiological investigation.** Isolates were inoculated to sheep blood agar and Sabouraud dextrose agar. White colonies which were roughtened and protruded from the surface, grew after five days of incubation at 35 °C in aerobic environments. The isolated microorganism was compatible with *Nocardia*. It was catalase-positive, oxydase-negative, lysozyme-positive and urease-positive.

DNA sequence analysis of clinical isolates using special laboratory equipment (RefGen) was accomplished using an ABI 3100 Genetic Analyzer (Applied Biosystems, USA). The sequencing data were analyzed using the BLAST system of the “National Center for Biotechnology Information (Bethesda, USA)” and these data had 100% concordance with *Nocardia otitidiscaviarum*. According to antibiotic susceptibility test results, the isolates were found to be resistant to cefotaxime (MIC >32 mg/L) and ampicillin-sulbactam (MIC >256 mg/L), and were found to be sensitive to amikacin (MIC =0.25 mg/L), imipenem (MIC =0.25 mg/L), linezolid (MIC =0.50 mg/L) and trimethoprim-sulfamethoxazole (TMP/SMX).

After three weeks of antimicrobial therapy, control MRI showed that the lesions sizes had increased. Antimicrobial therapy was changed to intravenous meropenem (6g/day) and trimethoprim (720mg/day)/sulfamethoxazole (3600mg/day). The lesions were drained by stereotactic craniotomy once a week consecutively for three weeks. Dural repair was performed after the drainage was completed. Intravenous medical treatment was continued for 8 weeks and the patient was discharged with only oral trimetophrim-sulfamethoxazole. After 4 weeks, at the control examination, CRP was 3 mg/L. Treatment was continued for 1 year and control MRI showed the regression of the lesions (Figure 3).

**DISCUSSION**

Nocardiosis is known as an opportunistic infection; the disease may occur rarely in immunocompetent patients. *N. otitidiscaviarum* is a relatively a rare cause of nocardiosis because of its low pathogenicity. Sytemic or disseminated disease is defined if two or more organs of the body are involved. Previously, disseminated infections were reported in cases with underlying malignancies, AIDS, organ transplantation, prolonged glucocorticoid therapy, chronic granulomatous disease [5]. In our case we reported the occurrence of systemic nocardiosis with CNS and retroperitoneal involvement due to *N. otitidiscaviarum* in an immunocompetent patient. Although her HIV test result was negative the fact that the CD4 lymphocyte count of the patient was low at 263/mm³ may have contributed to the onset of infection. A similar case was reported with multiple brain abscesses and CD4 lymphocytopenia recently [6]. Therefore, low CD4 lymphocyte count may be a predisposing factor for disseminated nocardiosis. The lower respiratory tract represents the most common site of infection in patients with nocardiosis. However, initial pulmonary focus usually cleared spontaneously, whereby the clinical symptoms of metastatic infections appearing first [1, 7]. In our patient, the symptoms of CNS infection were foreground; however the CNS involvement due to *N. otitidiscaviarum* is extremely rare. Only 12 cases with brain abscess were reported previously; three out of these were immunocompetent (Table 1) [7, 8].
<table>
<thead>
<tr>
<th>Case</th>
<th>Age, Sex</th>
<th>Sites of Infection</th>
<th>Underlying Disease</th>
<th>Choice and duration (days) of antimicrobial therapy</th>
<th>Surgery</th>
<th>Type of Surgical Operation</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Causey et al. [16]</td>
<td>54,M</td>
<td>CNS</td>
<td>Brain tumor</td>
<td>Ampicillin (NA)</td>
<td>No</td>
<td>-</td>
<td>F</td>
</tr>
<tr>
<td>Arroyo et al. [17]</td>
<td>45,M</td>
<td>CNS, disseminated</td>
<td>Renal tumor</td>
<td>Sulfadiazine (14), Streptomycin (11), Minocycline(4), Gentamicin(4)</td>
<td>No</td>
<td>-</td>
<td>F</td>
</tr>
<tr>
<td>Bradsher et al. [18]</td>
<td>63,M</td>
<td>CNS</td>
<td>No</td>
<td>TMP/SMX and Minocycline (NA)</td>
<td>Yes</td>
<td>Excision</td>
<td>F</td>
</tr>
<tr>
<td>Talwar et al. [19]</td>
<td>50,F</td>
<td>CNS</td>
<td>No</td>
<td>TMP/SMX and Gentamicin (NA)</td>
<td>Yes</td>
<td>Excision</td>
<td>F</td>
</tr>
<tr>
<td>Perez et al. [20]</td>
<td>31,F</td>
<td>CNS</td>
<td>HIV, drug abuser</td>
<td>Ciprofloxacin (30), Teicoplanin (30), Rifampicin (30), TMP/SMX (20), Netilmicin (20)</td>
<td>No</td>
<td>-</td>
<td>F</td>
</tr>
<tr>
<td>Hartmann et al. [21]</td>
<td>50,F</td>
<td>CNS and pulmonary system</td>
<td>Renal transplantation</td>
<td>Meropenem (42), Rifampicin (102), Ciprofloxacin (60)</td>
<td>Yes</td>
<td>Aspiration</td>
<td>S</td>
</tr>
<tr>
<td>Duran et al. [3]</td>
<td>21,M</td>
<td>CNS</td>
<td>Drug abuser</td>
<td>Imipenem (45) and TMP/SMX (45 days iv and 6 months orally)</td>
<td>Yes</td>
<td>Excision</td>
<td>S</td>
</tr>
<tr>
<td>Hemmersbach-Miller et al. [14]</td>
<td>44,M</td>
<td>CNS and Soft tissue</td>
<td>Renal transplantation and Diabetes mellitus</td>
<td>TMP/SMX (120), Amikacin (27), Metronidazole (27)</td>
<td>Yes</td>
<td>Aspiration</td>
<td>F</td>
</tr>
<tr>
<td>Lim et al. [22]</td>
<td>68,M</td>
<td>CNS</td>
<td>Diabetes mellitus and head injury</td>
<td>Meropenem (70), Amikacin (70), TMP/SMX (75 days iv and 12 months orally)</td>
<td>Yes</td>
<td>Aspiration and Excision</td>
<td>S</td>
</tr>
<tr>
<td>Bonnet et al. [23]</td>
<td>71,M</td>
<td>CNS and pulmonary system</td>
<td>Diabetes mellitus</td>
<td>Vancomycin (46), Ceftazidime (46), Gentamicin (46)</td>
<td>No</td>
<td>-</td>
<td>F</td>
</tr>
<tr>
<td>Pelaez et al. [2]</td>
<td>85,F</td>
<td>CNS and pulmonary system</td>
<td>COPD, coronary disease, hypertension</td>
<td>TMP/SMX (17), Imipenem (10), Linezolid (7)</td>
<td>No</td>
<td>-</td>
<td>F</td>
</tr>
<tr>
<td>Ishihara et al. [8]</td>
<td>79,F</td>
<td>CNS</td>
<td>No</td>
<td>Meropenem (7), panipenem/ betamipron (7), sulbactam/ ampicillin (14), cefozopran(14), TMP/SMX(34)</td>
<td>Yes</td>
<td>Aspiration</td>
<td>S</td>
</tr>
<tr>
<td>Present</td>
<td>69,F</td>
<td>CNS and retroperitoneal abscess</td>
<td>No</td>
<td>Meropenem (77), Amikacin (21), TMP/SMX (56 days iv and 10 month orally)</td>
<td>Yes</td>
<td>Aspiration</td>
<td>S</td>
</tr>
</tbody>
</table>

M: Male / F: Female / S: Survived / CNS: Central Nervous System / TMP/SMX: Trimethoprim/ sulfamethoxazole / NA: Not Accessible
N. otitidiscaviarum is known as a potentially zoonotic pathogen. The bacterium was first recognized in a guinea pig with an ear infection and N. otitidiscaviarum is a pathogenic species for dogs [2,9,10]. Our patient was a dog owner, without any evidence of infection in her dogs. Despite the occurrence of infection in various animals, the transmission of the disease from animals to humans has not been documented yet.

Nocardia spp. grow in non-selective media and the diagnostic yield is increased by the use of selective media such as Thayer-Martin agar. N. otitidiscaviarum has an ability to hydrolyze both hypoxanthine and xanthine is different from that of other species of Nocardia [4]. Colonial characteristics and cellular morphology are variable, and Nocardia spp. may be misidentified and confused with members of closely related genera. However, these techniques are laborious, time-consuming, and expensive and can take 1 to 3 weeks to accomplish. Sequence analysis of the 16S rRNA gene represents the gold standard to identify the species of Nocardia isolated [2].

The mainstay treatment for nocardial infections is antimicrobial therapy. Trimethoprim-sulfamethoxazole is the first choice of treatment especially for localized and mild forms of nocardiosis. It achieves high tissue concentrations in the lungs, brain, skin and bone. An imipenem and amikacin combination is suggested as initial therapy in cerebral nocardiosis and in the severely ill patients; however, even if in cerebral localization imipenem/cilastatin and amikacin combination are more effective than TMP/SMX, these combination can cause severe renal failure and deafness [1, 7]. Third generation cephalosporins such as ceftriaxone and cefotaxime are useful for brain abscess; they have excellent CNS penetration, but in our case N. otitidiscaviarum was found to be resistant to 3rd generation cephalosporins [11]. Combined treatment with amikacin also failed. Treatment was continued with TMP-SXT and meropenem. Isolates were sensitive to imipenem, but meropenem was the drug of choice because of the possible side effects of imipenem, such as convulsion. Meropenem has fewer side-effects than imipenem, has better penetration into the tissues and blood-brain barrier, and more active against N. otitidiscaviarum. In a multicenter study from Taiwan, 8 N. otitidiscaviarum isolates were 100% resistant to imipenem and 100% sensitive to amikacin, sulfamethoxazole and linezolid. Also, according to MIC values, meropenem was found more active than imipenem [12].

Nocardia abscesses may resemble brain tumors such as astrocytoma, metastasis and lymphoma. Stereotactic aspiration of the abscess is recommended for diagnosis, specific microbiological identification and anti-microbial sensitivity. For lesions larger than 2.5 cm in diameter surgical aspiration is recommended. Craniotomy and excision are reserved for lesions that enlarge after two weeks or fail to shrink after four weeks of antibiotic treatments [13]. Although the mortality of N. otitidiscaviarum brain abscess is high as 75%, reported cases with favorable outcome were treated with antibiotics combined with surgical procedures (Table 1) [14]. The recommended treatment for nocardial brain abscesses is high dose intravenous antibiotics for 3 to 6 weeks and then oral treatment for 12 months [15].

**CONCLUSION**

Infection by N. otitidiscaviarum is uncommon because of its low pathogenicity. Immunosuppression is the most important risk factor for nocardiosis but it may rarely occur in immunocompetent patients. Use of 16S rDNA gene sequencing for identification may allow us to determine rare etiologic agents properly such as N. otitidiscaviarum that cause brain abscess. Antimicrobial therapy combined with surgical procedures represents an optimal treatment of brain abscess due to N. otitidiscaviarum.

**Conflict of interest:** We confirm that there is no conflict of interest related to this article.

**REFERENCES**


