Case Report

Primary cutaneous nocardiosis caused by Nocardia nova in a kidney transplant recipient

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Nocardia nova is a rare aetiological pathogen for cutaneous nocardiosis. To the best of our knowledge, this is the first case of N. nova primary cutaneous infection in a kidney transplant recipient. Identification was performed using 16S rRNA and secA1 gene sequence analyses. The patient was not treated successfully by antibiotics alone. Surgical debridement was required for successful treatment.

Introduction

Species of the genus Nocardia, in the family Nocardiaceae, form a homogeneous cluster among the order Corynebacterineae, a suborder of Actinomycetales (Zhi et al., 2009). Nocardia species are a genus of aerobic actinomycete that are characteristically filamentous, branching, Gram-positive and alcohol-acid-fast at some stages of growth (Conville & Witebsky, 2007). Although Nocardia species are generally considered to have low pathogenicity, over one-third have been reported as human isolates (Conville & Witebsky, 2007). Clinical manifestations of nocardiosis range from cutaneous infection in a normal host to severe pulmonary or central nervous system or disseminated disease in an immunocompromised host (Brown-Elliott et al., 2006). The most common manifestation of nocardial infections is pulmonary nocardiosis. Extrapulmonary nocardiosis such as central nervous system, skin and disseminated infection is also relatively common (Yu et al., 2011; Wilson, 2012). Primary cutaneous nocardiosis is usually caused by trauma-related introduction of Nocardia species (Dodiuk-Gad et al., 2010). Unlike other forms of nocardiosis, primary cutaneous disease usually develops in immunocompetent hosts (Dodiuk-Gad et al., 2010).

We were only able to find five cases of Nocardia nova primary cutaneous infection in an English language literature search (Schiff et al., 1993; Shimizu et al., 2001; Inamadar et al., 2004; Antunes et al., 2012; Dhindra et al., 2012). However, three N. nova isolates from the reported cases were only identified phenotypically (Schiff et al., 1993; Shimizu et al., 2001; Inamadar et al., 2004), without confirmatory identification tests for Nocardia species. To the best of our knowledge, this is the first reported case of a primary cutaneous and soft tissue nocardiosis due to N. nova in a kidney transplant recipient.

Case report

A 51-year-old woman presented with erythematous and discharged skin lesions on the right foot, calf and thigh area. She had a history of end-stage renal disease secondary to hypertensive nephropathy 8 years ago, kidney transplant from cadaver-donor 4 years ago, fractures on the right second, third, fourth and fifth metatarsal bones due to trauma 6 months ago, and abrasion on her right knee 2 months ago. During the preceding 4 weeks, she had taken oral levofloxacin (750 mg day<sup>-1</sup>), but skin lesions worsened gradually. She had no specific event such as allograft rejection after kidney transplantation. She took the immunosuppressive agents tacrolimus (3 mg day<sup>-1</sup>), azathioprine (75 mg day<sup>-1</sup>) and methylprednisolone (8 mg day<sup>-1</sup>) without any problems.

On examination, the patient was afebrile and had a poorly demarcated erythema, swelling, tenderness and serous discharge on her right foot, calf and thigh. However, the patient was haemodynamically stable. Haematological investigations revealed a haemoglobin level of 12.5 g dl<sup>-1</sup>, a platelet count of 222 × 10<sup>9</sup> per litre, and a leukocyte count of 3.92 × 10<sup>9</sup> per litre. C-reactive protein was at 0.12 mg per litre. Erythrocyte sedimentation rate was at 2 m h<sup>-1</sup>. Renal and liver blood chemistry tests were within reference ranges, and anti-HIV antibody was negative. Skin biopsy revealed non-specific findings and
no evidence of fungal or tuberculous infection. She started receiving 2.0 g ceftriaxone day$^{-1}$ with outpatient-based follow-up. Her skin lesions improved gradually. After ceftriaxone therapy for 3 weeks, oral cefpodoxime (400 mg day$^{-1}$) was given. Her skin lesions improved to normal skin appearance, but she still felt some discomfort on her thigh. As fluctuations of the skin lesions on her thigh were observed, despite cefpodoxime therapy for 6 weeks, computed tomography was performed and multiple abscesses, the largest of which was 5.1 × 3.9 × 9.5 cm, were revealed (Fig. 1). The patient was taken quickly to the operating room on the day of admission and 20 × 7 cm infected mass was excised under spinal anaesthesia. The abscess material and swab specimens taken during the operation were sent for microscopy and culture. Specimens were plated on 5% sheep blood agar (BD Diagnostic Systems) and MacConkey agar (BD Diagnostic Systems) for bacterial culture, and inoculated onto Ogawa media (Union Lab) and the Bactec MGIT 960 system (BD Diagnostic Systems) using mycobacterial growth indicator tube (MGIT; BD Diagnostic Systems) media for mycobacterial culture. For fungal culture, a swab specimen was inoculated onto Sabouraud dextrose agar (BD Diagnostic Systems). After 4 weeks of incubation, small and wrinkled white colonies were observed on the Ogawa medium. Acid-fast staining with a modified Ziehl–Neelsen acid-fast stain revealed the bacteria to be partially acid-fast, arranged in branching filaments. No organisms were isolated from fungal, anaerobe and MGIT cultures. The identity of the organism was confirmed by sequencing of the 16S rRNA gene with primers 518F (5′-CCAGCAGCCGCGGTAATACG-3′) and 800R (5′-TACGAGGTTATCTAATCC-3′), sequencing was conducted using a Big Dye Terminator Cycle Sequencing kit (Applied Biosystems) and ABI PRISM 3730 genetic analyser (Applied Biosystems). All sequences were analysed using NCBI BLAST (National Center for Biotechnology Information Basic Local Alignment Search Tool) and RDB (Ribosomal Database Project). The 16S rRNA gene sequence (1428 bp) from our isolate showed 100% similarity with those of several N. nova strains (including GenBank accession numbers AB162789, AB162785, AF430030). The secA1 sequence (470 bp) showed the greatest similarity (99.57%) to that of N. nova (GU179115) and 98.71% similarity to that of Nocardia elegans (DQ360273). Based on our 16S rRNA and secA1 sequence analyses, we concluded that the case isolate was N. nova.

In vitro antimicrobial susceptibility testing was performed by broth microdilution assay (CLSI, 2011) according to the M24-A2 method of the Clinical and Laboratory Standards Institute (CLSI) (Table 1). The patient received 320 mg/600 mg day$^{-1}$ trimethoprim-sulfamethoxazole (SXT) for 4 months. Her skin lesions resolved completely without any complications.

### Discussion

In 1982, Tsukamura first described N. nova as a separate species with distinct phenotypic features from Nocardia asteroides and Nocardia farcinica (Yano et al., 1990). In 1990, DNA–DNA hybridization studies reported that N. nova had only 20% relatedness with N. farcinica and 39% relatedness with N. asteroides (Yano et al., 1990). In 1991, Wallace et al. determined that 17% of 223 clinical isolates

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (mg l$^{-1}$)</th>
<th>Susceptibility*</th>
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<tbody>
<tr>
<td>Ampicillin†</td>
<td>4</td>
<td>S</td>
</tr>
<tr>
<td>Amikacin</td>
<td>8</td>
<td>S</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>16/8</td>
<td>I</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>2</td>
<td>S</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;2</td>
<td>R</td>
</tr>
<tr>
<td>Erythromycin‡</td>
<td>0.5</td>
<td>S</td>
</tr>
<tr>
<td>Imipenem</td>
<td>1</td>
<td>S</td>
</tr>
<tr>
<td>Linezolid</td>
<td>2</td>
<td>S</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>2/38</td>
<td>S</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>&gt;8</td>
<td>R</td>
</tr>
<tr>
<td>Cefepime</td>
<td>8</td>
<td>S</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>1</td>
<td>S</td>
</tr>
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S, Susceptible; I, intermediate; R, resistant.

*Broth microdilution interpretive criteria are used as indicated in CLSI M24-A2.
†Broth microdilution interpretive criteria are used as indicated in CLSI M24-A.
‡Broth microdilution interpretive criteria for clarithromycin are used as indicated in CLSI M24-A since there are currently no CLSI interpretive criteria for erythromycin.
previously categorized as *N. asteroides* had a type III drug susceptibility pattern and belonged to the species *N. nova* (Wallace *et al.*, 1991; Brown-Elliott *et al.*, 2006). The only methods routinely available to separate *N. nova* strains from other members of *N. asteroides* were arylsulfatase activity at 2 weeks and antimicrobial susceptibility patterns, including susceptibility to ampicillin and erythromycin and resistance to amoxicillin-clavulanic acid. (Wallace *et al.*, 1991). However, these phenotypic characteristics are not always observed. Phenotypic testing has been rendered virtually useless for accurate speciation of *Nocardia* species because the number of phenotypic tests available in the clinical laboratory is too small and data on the phenotypic reactions in a given species are sparse (Brown-Elliott *et al.*, 2006; Convyle & Witebsky, 2007). It was also reported that some organisms identified as *N. nova* phenotypically and by heat-shock protein restriction fragment length polymorphisms actually belonged to other species in addition to *N. nova* (Convyle & Witebsky, 2007). CLSI guidelines for the identification of *Nocardia* species by DNA target sequencing recommended that *secA1* sequencing in addition to 16S rRNA sequencing provided better resolution for species level identification (CLSI, 2008).

The isolation frequency of *Nocardia* species varies according to geographical locations with a specific climate. In the USA, *N. asteroides* type VI and *N. farcinica* are distributed evenly throughout the nation, though *N. farcinica* is less prevalent than *N. asteroides*. In contrast, *N. nova* is less commonly isolated in the south-western USA, whereas *Nocardia brasiliensis* has a higher prevalence in the south-western and south-eastern regions (Saubolle & Sussland, 2003). Overall, the majority of human infections in the USA are caused by *N. asteroides* type VI, *N. brasiliensis*, *N. farcinica*, *Nocardia otitidiscaviarum* and *n. nova* (Saubolle & Sussland, 2003). In Taiwan’s subtropical climate, *N. brasiliensis* (47.8%) is the most common pathogenic *Nocardia* species, followed by *N. asteroides* (31.8%), *N. farcinica* (6.3%), *Nocardia flavourosea* (3.5%), *N. otitidis-caviarum* (2.7%) and *n. nova* (2.7%) (Tan *et al.*, 2010). In the temperate climate of northern Spain, the most prevalent *Nocardia* species is *N. nova* (29.6%), followed by *N. farcinica* (23.1%), *Nocardia cyriacigeorgica* (15.1%), *Nocardia abscessus* (12.4%) and *Nocardia carneae* (6.4%) (Larruskain *et al.*, 2011). Although the reasons for geographical differences in the isolation rate of *Nocardia* species are not clear, the optimal growth temperature of *Nocardia* species and regional climates may be major contributing factors.

The clinical presentation of nocardiosis varies with different *Nocardia* species and geographical locations. In the USA, members of the *N. asteroides* complex primarily cause pulmonary infection and, except for *N. nova*, are prone to cause extra-pulmonary dissemination. *N. farcinica* is often associated with disseminated infection whilst *N. brasiliensis* typically produces localized infection (Saubolle & Sussland, 2003). In the study by Larruskain *et al.* (2011), 95.2% (177/186) of clinical isolates of *Nocardia* species were recovered from respiratory samples, 1.6% isolates (3/186) were obtained from cutaneous abscesses (all *N. farcinica*), 1.6% from blood cultures (two *N. farcinica* and one *N. nova*), 1.1% (2/186) from urine (both *N. nova*) and 0.5% (1/186) from brain abscess (*N. abscessus*). Tan *et al.* (2010) reported that the major types of nocardial infection were primary cutaneous infection (56.6%), pulmonary infection (33.6%) and disseminated infection (7.1%). Although up to one-third of patients with nocardiosis are immunocompetent, the majority of nocardial infections occur in immunocompromised patients with suppressed cell-mediated immunity such as lymphoma, human immunodeficiency virus infection, and solid-organ or haematopoietic stem cell transplantation (Wilson, 2012). After skin inoculation, primary cutaneous nocardiosis usually presents as lymphocutaneous infection, superficial cellulitis or localized abscess, and in adults it usually involves the lower extremities (Brown-Elliott *et al.*, 2006).

Secondary cutaneous nocardiosis as a result of disseminated disease usually presents as pustules, abscesses and nodules (Dodiuk-Gad *et al.*, 2010). The relative frequency between the two types of cutaneous nocardiosis varies according to the prevalent *Nocardia* species; most of *N. brasiliensis* is isolated from immunocompetent individuals whereas *N. asteroides* is the predominant species in disseminated nocardiosis involving skin (Brown-Elliott *et al.*, 2006; Dodiuk-Gad *et al.*, 2010). *N. nova* is mainly associated with pulmonary and disseminated nocardiosis; it is a very rare cause of primary cutaneous or subcutaneous infection (Brown-Elliott *et al.*, 2006). In our case, it may be that the immunocompromised condition facilitated the invasion of *N. nova* via percutaneous trauma, and formed multiple huge subcutaneous abscesses.

*N. nova*, formerly known as *N. asteroides* type III drug susceptibility pattern, is susceptible to ampicillin, erythromycin, clarithromycin, linezolid, ceftriaxone, imipenem and amikacin, but intrinsically resistant to amoxicillin-clavulanic acid and ciprofloxacin (Wallace *et al.*, 1991; Brown-Elliott *et al.*, 2006). In our case, initial levofloxacin therapy may have contributed to the worsening of the patient’s clinical status because *N. nova* was resistant to levofloxacin. Her skin lesion seemed to respond well to ceftriaxone and cefpodoxime antimicrobial therapy. However, her localized abscess might have been an obstacle when treating with antibiotics alone. Although optimal antimicrobial treatment regimens have not been firmly established, sulfonamides and SXT have remained the treatment of choice for most *Nocardia* infections. Amikacin, minocycline, imipenem, third generation cephalosporins, and linezolid are considered as alternative agents (Brown-Elliott *et al.*, 2006; Dodiuk-Gad *et al.*, 2010). However, resistance rates to antimicrobial agents in *Nocardia* infections vary with *Nocardia* species (Larruskain *et al.*, 2011). Therefore *Nocardia* isolated from clinically significant infections should be identified to species level and undergo antimicrobial susceptibility testing to assist in
treatment decisions, and cases must be managed on an individual basis (Munoz et al., 2007). As in this case, if a patient has large-sized abscesses surgical drainage must be included in the treatment strategy, even though the abscess size cannot be determined.

In conclusion, we report the first case of primary cutaneous and subcutaneous multiple abscesses caused by \textit{N. nova} in a kidney transplant recipient who was treated successfully with surgical drainage and antibiotics.

\textbf{References}


