Case Report

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First isolation of *Nocardia grenadensis* from a clinical sample

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Introduction: *Nocardia* species have a worldwide distribution and are commonly found as saprophytes of soil or water. However, they can cause human disease especially in immunocompromised patients, with respiratory infection being the most frequent clinical manifestation of the disease. The aim of the present work was to describe the first clinical isolation of *Nocardia grenadensis* from the sputum of a patient from the north of Spain suspected of having tuberculosis.

Case Presentation: In November 2012, a chest X-ray showed a mass in the left upper lobe of the lung of a 75-year-old man with persistent cough with bloody expectoration. The patient was sent to the outpatient pulmonology consultation for further studies. In the sputum sent for mycobacterial study, a *Nocardia* isolate was present that was identified by sequencing a 1440 bp fragment of the 16S rRNA gene as *N. grenadensis* at the Special Bacteriology Reference Laboratory at the Centers for Disease Control and Prevention (USA).

Conclusion: The clinical significance of *N. grenadensis* in the sputum of this patient was uncertain, as symptoms of persistent cough with haemoptysis in the absence of other identified causes suggested the possible involvement of *N. grenadensis* in a bronchial lung infection. However, the spontaneous resolution of symptoms without specific antimicrobial treatment pointed to the possibility of a transient colonization. To the best of our knowledge, this is the first evidence in the literature of the isolation of *N. grenadensis* in humans.

Keywords: haemoptysis; persistent cough; respiratory infection; whitish expectoration.

Introduction

Bioprospecting and activity directed toward the selection of micro-organisms with economic value is under permanent development. In this regard, there has been significant work on the study of the diversity and biology of the genus *Nocardia* in natural habitats (Albuquerque de Barros *et al.*, 2003). The genus *Nocardia* has an important role in the biotechnology and pharmaceutical fields due to the production of antimicrobials and enzymes for medical and industrial use (Mukesh, 2014).

In 1995, only 15 species were classified within the genus *Nocardia*. At present, this genus comprises >100 species,

the great majority of them (>85 %) having been identified by molecular methods (<http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi>).

Although less than half of the validly named *Nocardia* species have been characterized as human pathogens, new pathogenic species causing clinical infections are continually being recognized and reported (Ambrosioni *et al.*, 2010; Jannat-Khah *et al.*, 2010). The development of molecular techniques and increasing access to diagnostic laboratories has greatly eased the identification of *Nocardia* species. Among them, sequencing of different constitutive genes, especially the 16S rRNA gene, has become one of the most widely accepted methods for accurate species identification (CLSI, 2008).

To date, *Nocardia grenadensis* has been described on two occasions, both of them from environmental sources

Abbreviation: COPD, chronic obstructive airways disease.

The GenBank/EMBL/DDBJ accession number for the sequence of the 1440 bp fragment of the 16S rRNA gene from the *Nocardia* isolate is KT254140.

(Kämpfer *et al.*, 2012; Wilson, 2013). The aim of the present work was to describe the first isolation of *N. grenadensis*, identified by 16S rRNA gene sequencing, from the sputum of a patient from the north of Spain suspected of having tuberculosis.

Case Report

N. grenadensis was isolated from a bronchial secretion sample obtained after spontaneous expectoration from the sputum of a 75-year-old man with a history of previous prostate adenocarcinoma, arterial hypertension, hyperlipidaemic disorder and osteoarthritis. The patient had also a lung mass in the lung left upper lobe with a pseudonodule in the first left rib. The patient was a non-smoker, did not have any chronic obstructive pulmonary disease (COPD) and had no recent history of contact with a tuberculosis patient nor any travel abroad in the previous year.

Investigations

In April 2012, the patient attended his primary care physician because of persistent cough and whitish expectoration, sometimes with haemoptysis. In November 2012, the patient continued with cough and expectoration with haemoptysis, and a chest X-ray showed a lung mass in the left upper lobe which was interpreted as a pseudonodule in the first left rib. In December 2012, sputum samples were collected for microbiological cultures (bacterial and tuberculosis) and pathological analysis. A culture positive for *N. grenadensis* and pathology results negative for neoplastic cells, showing abundant macrophages and squamous metaplasia cells, were obtained. Both acid-fast stain and culture were negative for *Mycobacterium*.

In January 2013, the patient attended his general practitioner still with cough. The investigation of *Nocardia* in a new sputum collected in February 2013 was negative. The chest radiograph performed showed no specific abnormalities, although the patient's symptoms persisted for >6 months. The pseudonodule observed in the first chest X-ray was considered as an overlying rib. Finally, without antimicrobial treatments (to our knowledge), the symptoms disappeared.

N. grenadensis showed a good growth of white colonies of 1–2 mm size and matt appearance after 48 h incubation at 35 °C on blood agar. The colony appearance was circular with raised elevation and filamentous edges. The catalase test results were positive.

The isolate was submitted to the Special Bacteriology Reference Laboratory at the Centers for Disease Control and Prevention (USA) for identification. A 1440 bp fragment of the 16S rRNA gene was amplified by PCR and sequenced according to methods described previously (Lasker *et al.*, 2011). The resulting sequence was submitted to GenBank. Related gene sequences in GenBank were identified using BLASTN (<https://www.ncbi.nlm.nih.gov/blast/>). The best match was found to be *N. grenadensis*

GW5-5797^T, which shared 100 % similarity with the isolate sequence (1440/1440 bp).

Treatment

N. grenadensis antimicrobial susceptibility testing was performed by broth microdilution according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2011), and showed non-susceptibility to amoxicillin/clavulanic acid (MIC=16 µg ml⁻¹) and clarithromycin (> 16 µg ml⁻¹). The *N. grenadensis* showed *in vitro* susceptibility to ampicillin (< 4 µg ml⁻¹), amikacin (< 4 µg ml⁻¹), cefepime (2 µg ml⁻¹), cefotaxime (1 µg ml⁻¹), ceftriaxone (1 µg ml⁻¹), ciprofloxacin (1 µg ml⁻¹), doxycycline (0.25 µg ml⁻¹), imipenem (< 1 µg ml⁻¹), linezolid (< 1 µg ml⁻¹), minocycline (< 1 µg ml⁻¹), moxifloxacin (1 µg ml⁻¹), tobramycin (< 1 µg ml⁻¹) and trimethoprim/sulfamethoxazole (< 2/38 µg ml⁻¹). The patient was not specifically treated for the *Nocardia* isolated and continued with the usual treatment for his pathologies.

Outcome and Follow-up

The patient continued with dry cough and expectoration with haemoptysis for at least 6 months beyond the *Nocardia* isolation, a period during which the patient had a follow-up at radiological, pathological and microbiological levels, including three more sputum cultures, without any pathogenic micro-organism detected in any of them. No antibiotic treatment was prescribed during this time period. In June 2013, the patient was discharged from the Pneumology Department by absence of bronchopulmonary pathology.

Discussion

Respiratory infection is the most frequent clinical manifestation of nocardiosis, especially in COPD patients because infection normally takes place via the respiratory tract. However, *Nocardia* can also be found infecting the central nervous system, skin and other locations (Brown-Elliott *et al.*, 2006). The risk of nocardiosis in immunocompromised patients is 140- to 340-fold higher than in the non-immunosuppressed population (Filice, 2005).

The identification of *Nocardia* isolates to the species level has traditionally been based on the assessment of growth characteristics, antibiotic resistance and biochemical testing (Brown-Elliott *et al.*, 2006). However, with the growing taxonomy of the genus, the utility of traditional biochemical methods for the accurate identification of some *Nocardia* species is no longer reliable. Currently, the analysis of the sequence of certain genes is considered the best method for correct *Nocardia* species identification and could be a satisfactory alternative to DNA–DNA hybridization as the gold standard for defining new species (Clarridge, 2004). In one study that analysed the utility of sequencing five different genes (16S rRNA, *secA1*, *gyrB*, *hsp65* and *rpoB*) to identify different *Nocardia* species,

it was highlighted that the identification achieved using the combined sequences differed from identifications obtained by analysing individual gene sequences (McTaggart *et al.*, 2010).

Our isolate was identified as *N. grenadensis* by 16S rRNA sequencing. However, when we analysed the *secA1* gene sequence, the GenBank best match (BLASTN) was the *secA1* gene of *Nocardia sienata* with 96.8 % similarity. This result, which initially could be regarded as a discrepant identification, was due to the absence of the *secA1* sequence of *N. grenadensis* in GenBank. It always has to be taken into account that when a gene sequence is being analysed it might not be included in the database searched, especially if a 100 % similarity is not obtained and, as in the case herein described, the genes under study may belong to a relatively newly described species. *N. grenadensis* was originally isolated in 2012 from a sand sample collected at 5 m depth from the Caribbean Sea near Grenada (Kämpfer *et al.*, 2012). A *N. grenadensis* isolate was also identified 1 year later during the characterization of organisms collected from different soils in the Great Smoky Mountains National Park North Carolina (USA) (Wilson, 2013). The patient described here did not have a history of travel in the year previous to the *N. grenadensis* isolation. This suggests that, considering the different regions where *N. grenadensis* has been isolated, it is a species that may be ubiquitous.

The clinical significance of *N. grenadensis* in the sputum of this non-COPD patient is uncertain. Symptoms of persistent cough with whitish expectoration, including haemoptysis, in the absence of other identified causes suggested the possible involvement of *N. grenadensis* in a bronchial lung infection. However, the absence of altered radiology and blood parameters of infection together with the apparent spontaneous resolution of symptoms could not exclude the possibility that this microbiological finding represented a transient colonization.

In conclusion, the present work shows that gene sequencing offers a rapid and accurate way for identification of even very uncommon *Nocardia* species. To the best of our knowledge, this is the first report in the literature of the isolation of *N. grenadensis* in humans.

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