Introduction
Nocardiosis is a localized or disseminated infection caused by Nocardia spp., aerobic actinobacteria that can affect the skin, lung, brain, eye, joint, bone, heart and other organs. It has a worldwide incidence, does not exhibit ethnic predominance, seems to be more frequent in patients aged 30–40 years (McNeil & Brown, 1994), and is two to three times more common in males than in females (Yildiz & Doganay, 2006; Yildiz et al., 2005).

Nocardiosis can occur in association with a deficiency in cellular immunity (Pintado et al., 2003; Hamadani et al., 2008) – such as human immunodeficiency virus (HIV) infection, other immunodeficiency syndromes, or chronic use of systemic steroids – or in immunocompetent hosts. In the immunocompromised patient the infection starts in the lung, before spreading haematogenously to the central nervous system (CNS) or other organs. In contrast, in the immunocompetent patient the disease typically takes a chronic course in a single organ or region (Ambrosioni et al., 2010). The occurrence of Nocardia colonization without manifestation of disease is subject of increasing interest (Severo et al., 2013; Tremblay et al., 2011).

The micro-organism is ubiquitous in the environment, namely in soil, dust, water (both fresh and salt), faeces and decaying vegetation (McNeil & Brown, 1994); thus, most human Nocardia infections are acquired by inhalation, while a minority result from traumatic percutaneous inoculation. It does not appear to be transmitted from person to person and is rarely nosocomial, although cases of infection in hospital wards have been described (Saubolle & Sussland, 2003).

In this work we describe five cases of disseminated nocardiosis, diagnosed between April 2010 and January 2012, in Centro Hospitalar S. João, Porto, Portugal.

Description of cases
Case 1: Nocardia farcinica
A 75-year-old female came to the hospital with progressive asthenia and dyspnoea. She had a previous history of type 2 diabetes mellitus, hypertension, dyslipidaemia, and chronic thrombocytopenia for which she had been medicated with prednisolone 40 mg day\(^{-1}\) for over 1 year.
On admission, she was conscious, collaborative, oriented and did not exhibit neurological signs. A chest radiograph showed no lesions, but a thoracic computerized tomography (CT) scan evidenced a well-circumscribed 10 mm nodule in the left upper lung lobe and incipient bronchiectasis; no mediastinal or hilar lymphadenopathy was recognized. She was discharged and continued prednisolone 40 mg day⁻¹.

Two months later, she returned with gait imbalance, without fever or respiratory complaints. The brain CT scan showed a space-occupying right fronto-parietal lesion, with oedema and mass effect, whereas a pulmonary CT scan revealed a significant size reduction of the previously observed nodule. As brain magnetic resonance imaging (MRI) evidenced multiple fronto-parietal–temporal lesions, suggestive of pyogenic abscesses, surgical drainage was performed and combined antibiotic therapy with ceftriaxone and metronidazole was initiated. On the fourth day, a strain of *Nocardia* spp. was isolated in the pus culture, and antibiotic therapy was changed to imipenem and trimethoprim/sulfamethoxazole. Owing to lack of brain lesion improvement, amikacin was added at the fourth week. She completed 3 months of combined antibiotic therapy and 14 months of consolidation therapy with trimethoprim/sulfamethoxazole as outpatient.

**Case 2: most closely related to Nocardia brasiliensis**

A 56-year-old female with a skin lesion on the right leg was observed. She had a previous history of type 2 diabetes mellitus, hypertension and thrombocytopenia with treatment with prednisolone 1 mg kg⁻¹ day⁻¹ for the past 4 months. She had been admitted to another hospital with the diagnosis of lung abscess a few weeks before. At that time, a chest CT scan evidenced a right peri-hilar densification with a 22 mm cavity extending dorsally and additional nodular dense areas, adjacent to the middle and lower lobes. Bronchoscopy revealed no macroscopic changes and bronchoalveolar lavage (BAL) was negative for micro-organisms and neoplastic cells. She was empirically treated with a third generation cephalosporin and a macrolide and discharged.

In the current observation, an ultrasound of the leg lesions showed a 54 × 25 mm heterogeneous fluid collection, distally in the right biceps femoris muscle, whose drained pus was sent to culture.

Upon 4 days in culture, a *Nocardia* sp. was isolated and the patient was called back. She reported lesion worsening, severe pain on walking, and episodes of headache and dizziness. The patient denied cough, dyspnoea, or fever, and the physical examination did not evidence neck stiffness or neurological deficits. Thoracic CT revealed a condensation of the outer segment of the middle lobe, suggesting infection. Treatment with intravenous trimethoprim/sulfamethoxazole and amikacin was initiated and continued for 4 weeks, when chest imaging documented a favourable evolution. Because of a platelet count of 15 000 mm⁻³, prednisolone was adjusted to 20 mg day⁻¹ on discharge. She completed 13 months of trimethoprim/sulfamethoxazole treatment as outpatient.

**Case 3: Nocardia nova**

A 48-year-old male with previous history of HIV infection was admitted to the hospital with fever, mild productive cough, left pleuritic chest pain, minimal effort dyspnoea, cervical discomfort (he said it was difficult to hold his head up) and mild left arm paraesthesia. Headache, fever, relevant skin lesions or palpable lymph nodes were absent. The patient had been medicated with highly active antiretroviral therapy. In addition, he had a stage IV lung adenocarcinoma with cerebellar metastasis undergoing treatment with radiotherapy, chemotherapy and prednisolone, 40 mg day⁻¹, for over a year. Because of a previous history of trimethoprim/sulfamethoxazole allergy, prophylaxis with atovaquone was indicated.

Lymphocyte CD4⁺ count was 215 cells mm⁻³. Chest radiography showed multiple cavitary lung lesions. The patient began treatment with piperacillin/tazobactam and ciprofloxacin; ceftriaxone was added later owing to lack of clinical improvement. After 4 days culture of bronchial secretions, *Nocardia* sp. was isolated, which prompted treatment with meropenem and amikacin. After 15 days, the patient left the hospital ward against medical advice, but he returned 2 weeks later, complaining of inability to support his head (he needed to hold his chin with his hand to maintain cervical extension), neck pain and decreased left arm strength. Chest radiography revealed C6 fracture and MRI showed extradural empyema of C4 to C7. Corpectomy of C6 and C7 and arthrodesis of C5 to T1 with iliac bone graft and anterior cervical plate fixation were performed, and trimethoprim/sulfamethoxazole desensitization was begun. Later, a thoracic–abdominal CT scan revealed lung lesions worsening, rib destruction in relation to the superior right lobe, multiple nodular lesions in the liver and the posterior renal space adherent to peritoneum, infiltration of right adductors, and one thyroid nodule. Blood, bronchial secretions, cervical vertebra and thyroid pus cultures were positive for the same strain of *Nocardia*, whereas pus drained from the retroperitoneum was negative. After 1 month of amikacin and imipenem, the patient refused additional treatment owing to nausea and vomiting. Two weeks later he accepted imipenem, amikacin and ceftriaxone, which continued for 44 days, when he abandoned medical surveillance.

**Case 4: Nocardia farcinica**

A 42-year-old male came to the hospital complaining of a progressively larger and painful 10 cm swelling of the left arm, diagnosed as an abscess, unsuccessfully medicated
with ciprofloxacin for 15 days. He had a previous history of traumatic splenectomy, silicosis aggravated by bronchitis and pulmonary tuberculosis, and was medicated with prednisolone 20 mg day⁻¹ for 1 year.

On admission, pus collected from the abscess drainage was sent for culture and the patient was discharged with amoxicillin/clavulanate.

One week later he returned with a similar lesion in the right thigh and, at the same time, a Nocardia sp. was isolated from the pus culture. Brain CT scan showed an abscess-like image in the left parietal region. Lung CT scan showed randomly distributed nodules that, although non-specific, were interpreted as foci of Nocardia infection. The brain MRI confirmed a left parietal region abscess. Surgical drainage was performed and pus cultures were negative. The patient was medicated with meropenem, amikacin and trimethoprim/sulfamethoxazole for 2 months, and both lung and brain lesions sizes were reduced. He completed 12 months of consolidation therapy with trimethoprim/sulfamethoxazole as outpatient.

**Case 5: Nocardia pseudobrasiliensis**

A 41-year-old male with previous history of hypertension, dyslipidaemia and tabagism, with chronic alcoholic hepatic disease, under treatment with prednisolone 40 mg day⁻¹ for over a year, came to the hospital with asthena, cough, brownish bronchial secretions, oedema of both legs, increased abdominal girth and mild rectal haemorrhage. On physical examination, his temperature was 38°C and he had polypnoea, wheezing in the right hemithorax, jaundice of the skin and sclera, and hepatomegaly. Chest radiography demonstrated condensation of the right apex, and thoracic CT showed a cavitated thick-walled mass 87×61 mm in size in the superior right lobe. BAL cytology was negative for tumoral cells, and on the fourth day a strain of Nocardia spp. was isolated on culture. After 1 month of imipenem and intravenous cotrimoxazole, he was discharged with amoxicillin/clavulanate.

Two months later, he returned to the hospital with panophthalmitis of the right eye. CT scan of the orbits showed a right eyeball abscess and peri-orbital cellulitis with post-septal extension but absence of brain lesions. Emergency eye enucleation was performed and eyeball cultures were positive for the same strain of Nocardia spp. He was treated with imipenem and intravenous trimethoprim/sulfamethoxazole for 9 weeks. Subsequent bronchial secretion cultures were negative. His second admission was aggravated by cytomegalovirus pneumonia, which required invasive ventilation. The patient died on the 63rd day.

**Microbiology**

Different biological samples were collected, namely drainage of brain abscess, biopsy of skin lesions, aspirate of a thyroid nodule, biopsy of the cervical vertebrae, BAL and enucleation of the eye, and sent to the microbiology laboratory. After macroscopic examination, smears were prepared and Gram-stained. The skin lesion aspirates of cases 2 and 4 showed Gram-positive thin branched rods (Fig. 1a). They were then stained with modified Kinyoun acid-fast stain, which showed branched rods with partial acid resistance (Fig. 1b).

Subsequent cultures were made on blood and chocolate agar, and incubated under 5% CO₂ at 37°C. By day 4, white colonies with a chalky appearance presented on blood agar. In aged cultures of some species, the white colour turned to orange.

On day 12, the colonies’ features varied considerably. Some exhibited a star-like outline and an elevated centre (N. farcinica), others were a cream colour, with irregular edge and a small central pit (N. brasiliensis), others had a smooth round boundary (N. nova), and one had a clover-like appearance and contained a small central pit (N. pseudobrasiliensis) (Fig. 2a–d). These differences were not unexpected. Nocardia colonies exhibit large morphological variety, depending on the medium and the incubation temperature used. Colony smears stained by the Gram and Kinyoun methods confirmed the observations of the earlier smears.

In our molecular biology laboratory the genus of each isolate was identified by real-time PCR combined with melting-curve analysis (Alfaresi & Elkosh, 2006). NG1, a forward primer complementary to positions 966 to 982 on the antisense strand, and NG2, a reverse primer complementary to positions 386 to 405 on the sense strand, were used (Laurent et al., 1999). The formation of a double-stranded PCR product, about 596 bp long, was detected by SYBR Green I.

![Fig. 1. Direct Gram smear (a) and modified Kinyoun acid-fast stain (b) of Nocardia.](http://jmmcr.sgmjournals.org)
Currently, the identification of Nocardia to the species level and their susceptibilities are epidemiologically important (McTaggart et al., 2010).

For species identification, a 597 nt fragment of the 16S rRNA gene and around 441 nt of the hsp65 gene were PCR amplified according to the protocols established by Rodrı´guez-Nava et al. (2006), except for case 2, for which a 1372 nt fragment of the 16S rRNA gene was used. The DNA of each clinical isolate was amplified by using both genes in order to confirm the species identification as recommended (Brown-Elliott et al. 2006; Schlaberg et al. 2008; CLSI, 2008). The analysis of the sequences was done with BLAST software [nucleotide–nucleotide BLAST (http://www.blast.ncbi.nlm.nih.gov/Blast.cgi)]. The results of the identification at species level according to the percentage similarity (the minimum percentage similarity requested was 99%) of our clinical isolates with the sequences of the type strains present in the GenBank database can be found in Table 1.

Antimicrobial susceptibility testing was performed and the results analysed using the disc diffusion method on

### Table 1. Antimicrobial susceptibility and molecular identification of the case series

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
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<tbody>
<tr>
<td>ID</td>
<td>N. farcinica</td>
<td>Most closely related to N. brasiliensis</td>
<td>N. nova</td>
<td>N. farcinica</td>
<td>N. pseudobrasiliensis</td>
</tr>
<tr>
<td>Susceptibility</td>
<td>amc, ak, moxi, lzd, sxt, cip, lev, mino</td>
<td>amc, tob, ak, lzd, sxt, moxi, mino</td>
<td>imi, sxt, cfx, cfx, ctf, cro, cef, ak, lzd, clari</td>
<td>amc, imi, ak, lzd, sxt, moxi, mino, doxi</td>
<td>imi, ct, cef, ak, lzd, moxi, doxi, cfx</td>
</tr>
<tr>
<td>Resistance</td>
<td>cfx, ctf, cro, cef, imi, tob, doxi, clari</td>
<td>cfx, ctf, cro, cef, cef, lev, doxi, clari, imi*</td>
<td>amc, tob, cef, lev, moxi, doxi, mino</td>
<td>cfx, ctf, cro, cef, tob, cef, lev, clari</td>
<td>amc, cef, tob, cef, lev, moxi, clari</td>
</tr>
<tr>
<td>Sequence similarity to 597 nt 16S RNA gene (%)</td>
<td>99.091</td>
<td>99.321</td>
<td>100.002</td>
<td>99.413</td>
<td>100.001</td>
</tr>
<tr>
<td>Sequence similarity to hsp65 gene (%)†</td>
<td>99.091</td>
<td>99.321</td>
<td>100.002</td>
<td>99.413</td>
<td>100.001</td>
</tr>
</tbody>
</table>

Abbreviations: ak, amikacin; amc, amoxicillin/clavulanate; cef, cefepime; cfx, cefuroxime; cip, ciprofloxacin; clari, clarithromycin; cro, ceftriaxone; cft, cefotaxime; doxi, doxycyclin; imi, imipenem; lev, levofloxacin; lzd, linezolid; mino, minocyclin; moxi, moxifloxacin; sxt, trimethoprim/sulfamethoxazole; tob, tobramycin.

* Intermediate susceptibility.
† 1327 nt fragment of 16S RNA gene.
‡ Size of hsp65 gene fragment: 1441 nt; 3439 nt; 339 nt.
cation-supplemented Mueller–Hinton medium according to the Clinical and Laboratory Standards Institute standard M24-A2 (CLSI, 2011) and Société Française de Microbiologie guidelines (CA-SFM, 2013; Rodriguez-Nava et al., 2014). The drugs tested were amoxicillin/clavulanic acid (20/10 μg), ceftriaxone (30 μg), cefuroxime (30 μg), cefotaxime (30 μg), cepfime (30 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), linezolid (30 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), amikacin (30 μg), tobramycin (10 μg), imipenem (10 μg), minocycline (30 μg), moxifloxacin (5 μg), doxycycline (30 μg) and clarithromycin (15 μg) (Bio-Rad). The susceptibility results for these species are displayed in Table 1.

**Discussion**

The diagnosis of nocardiosis is difficult, because clinical signs and symptoms are non-specific and imaging is not pathognomonic (Yildiz & Doganay, 2006). The most feared course of the infection is disseminated nocardiosis, which involves at least two organs. In most cases the lung is implicated because inhalation is the primary route of exposure. The CNS is the second most common site of systemic dissemination (25–45%), followed by the skin (10%) and other less frequent sites (Dodiuk-Gad et al., 2010). The most frequently involved species are *N. brasiliensis*, *Nocardia otitidiscaviarum*, *Nocardia transvalensis*, *N. farcinica* and *N. nova* (McNeil & Brown, 1994), but other species have also been recognized as causal agents (Brown-Elliott et al., 2006).

All patients in our series were under corticosteroid therapy and had additional immunosuppression-prone conditions, namely idiopathic thrombocytopenic purpura, acquired immunodeficiency syndrome, asthma, chronic hepatic disease or diabetes mellitus. One of our patients presented with CNS involvement (case 1) due to *N. farcinica*, two had cutaneous disease (cases 2 and 4) caused by *N. brasiliensis* and *N. farcinica* respectively, the third case had a vertebral abscess caused by *N. nova*, and the fifth case an eye infection caused by *Nocardia pseudobrasiliensis*. The major condition in our series was immunosuppression secondary to corticosteroid therapy, which emphasizes once again that nocardiosis should be part of the differential diagnosis whenever an immunocompromised patient presents with skin, brain or pulmonary lesions.

Usually imaging is non-specific, pleomorphic and not pathognomonic (Yildiz & Doganay, 2006). For this reason microbiological examination, with appropriate evaluation of the sample for smear and culture, became the main method to ascertain the diagnosis of *Nocardia* infection (Wilson, 2012). In fact, in all cases, it was the laboratory that successfully isolated and identified the causal agents. To accomplish this task, extra culture time is required for *Nocardia* growth and isolation to occur, as colonies take longer than the usual 48–72 h.

Finally, identification to the species level was accomplished by molecular procedures (McTaggart et al., 2010; Brown-Elliott et al., 2006). Biochemical tests are time consuming and often inconsistent (Brown-Elliott et al., 2006). Molecular techniques, like 16S rRNA gene sequencing, were considered until very recently the ‘gold standard’ for bacterial identification, but they failed to discriminate between many species of *Nocardia*. The association of 16S rRNA and *hsp65* gene sequencing (Rodriguez-Nava et al, 2006) seems promising in the correct differentiation of *Nocardia* species. This methodology was used for isolate identification in the current study.

The correct and timely diagnosis of *Nocardia* infection and the institution of proper therapy can be life-saving (Torres et al, 2000), but a delay is not uncommon. The exact identification of the species is important, since different species have different susceptibility profiles. The antimicrobial susceptibility tests are also crucial; our two isolates of *N. farcinica* showed some differences in their drug susceptibility patterns (Table 1) and this information is critical to adjust antibiotic treatment. However, while waiting for the results, empirical treatment must start. For the last 60 years sulphonamides have been the therapy of choice for this disease (Ambrosioni et al., 2010). Trimethoprim/sulfamethoxazole (cotrimoxazole) has been the drug most often employed, owing to its pharmacokinetics and to particularly good CNS penetration. In patients with disseminated infection, however, monotherapy is not recommended (Ambrosioni et al., 2010) and the combination of amikacin with imipenem appears to be superior to other antibiotics (Gombert et al., 1990). However, most *N. brasiliensis* strains are resistant to imipenem and this must be considered when choosing treatment. Actually, in our study, an isolate from case 2 presented an intermediate susceptibility to this antimicrobial. Other antimicrobials with clinical benefit are extended spectrum cephalosporins, fluoroquinolones (especially moxifloxacin, more active than ciprofloxacin), clindamycin, erythromycin, ampicillin, aminoglycosides (particularly amikacin), tetracycline (including minocycline) and linezolid.

Regarding our patients, the *in vitro* susceptibility patterns were in accordance with the *in vivo* results for cases 1, 2, 3 and 4. This agreement was not observed in case 5, where cotrimoxazole had resistance *in vitro* but was, apparently, effective *in vivo*.

Our series corroborates the enhanced risk of nocardiosis among immunocompromised patients, the need to extend culture time when nocardiosis is suspected, the importance of employing molecular diagnostic tools for the identification of the species and the significance of antimicrobial susceptibility tests in order to complete the assessment of *Nocardia* species under study, and confirms the importance of performing a careful CNS assessment and excluding other infectious foci upon diagnosis of nocardiosis.

**Acknowledgements**

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References


