Clinical and microbiological features of nocardiosis 1997–2003

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Nocardiosis has been believed to be caused by the members of the Nocardia asteroides complex and the Nocardia brasiliensis species. However, recent advances in genotypic identification have shown that the genus exhibits considerable taxonomic complexity and the phenotypic markers used in the past for its identification can be ambiguous. The aim of this study was to assess the species distribution of Nocardia isolates and to determine whether there are differences in pathogenicity or antimicrobial susceptibility between the different species identified. Nocardia isolates obtained over a 7 year period were retrospectively reviewed. The isolates were identified genotypically, their antibiotic susceptibility was tested and the clinical data of the 27 patients were retrieved. Eight different Nocardia species were identified: Nocardia farcinica (n=9), Nocardia abscessus (n=6), Nocardia cyriacigeorgica (n=6), Nocardia otitidiscaviarum (n=2), Nocardia nova (n=1), N. nova complex (n=1), Nocardia carneae (n=1) and Nocardia transvalensis complex (n=1). All species were susceptible to co-trimoxazole but different patterns of susceptibility to other agents were observed. All patients had active comorbidities at the time of infection. A total of 19 patients were immunosuppressed, due to human immunodeficiency virus infection, chronic corticosteroid therapy, immunosuppressive therapy or haematological malignancies. Six patients displayed a Charlson comorbidity index score above 4. Global mortality was 50 % while attributable mortality was 34.6 %. Patients infected with N. farcinica – the most resistant species – had the highest Charlson index score and the highest mortality rate. Accurate identification of the species and susceptibility testing of Nocardia isolates may play an important role in diagnosis and treatment.

INTRODUCTION

Nocardia species are soil-borne aerobic actinomycetes with a worldwide distribution. Nocardia can cause pulmonary or disseminated infections or, more rarely, subcutaneous actinomycotic mycetomas by direct skin inoculation. Nocardiosis is considered to be an opportunistic infection and it most commonly presents as pulmonary disease, but clinical diagnosis of nocardiosis is difficult, due to non-specific manifestations (Saubolle & Sussland, 2003).

This group of organisms was classically identified from microscopic morphology and phenotypic characterization. Identification of the isolates using conventional phenotypic assays is laborious and time-consuming, and considerable expertise is required to accurately identify newer species (Wallace et al., 1991). The taxonomy of the genus Nocardia has undergone several reclassifications in recent years. Molecular identification of new species has expanded our knowledge about the genus and contributed to elucidating its taxonomy (Brown-Elliott et al., 2006; Patel et al., 2004). More than 50 species have now been characterized by phenotypic and molecular methods. However, not all of these species have been subjected to the same level of analysis (Brown-Elliott et al., 2006). Approximately 16 species have been implicated in human infections (Roth et al., 2003; Saubolle & Sussland, 2003), but the geographical prevalence of each one throughout the world may differ dramatically and some are uncommon. It is presently uncertain whether there are clinically meaningful differences between Nocardia species regarding the spectrum...
of the disease, antimicrobial susceptibility patterns and consequently optimal therapy.

The aim of this study was to assess the species distribution of Nocardia isolates in a general hospital over a 7 year period, and to investigate whether the different species identified differed in pathogenicity or antimicrobial susceptibility.

**METHODS**

**Study setting, patients and isolates.** We retrospectively reviewed all patients diagnosed with nocardiosis over a 7 year period (January 1997–November 2003) in a 600 bed university hospital in Barcelona, Spain. Identification as a Nocardia sp. was considered when an isolate was a branched Gram-positive bacillus, acid-fast by the modified Kinyoun stain and formed aerial vegetative filaments. Twenty-seven clinical isolates and two control strains (Nocardia asteroides ATCC 19247 and Nocardia otitidiscaviarum ATCC 14629) were studied. A 500 bp 16S rDNA fragment was amplified and sequenced with specific primers (Cloud et al., 2004). The resulting DNA sequences were compared to GenBank and RIDOM database sequences (Mellmann et al., 2003). Nocardia abscessus and Nocardia asiatica were differentiated by nitrate reduction and decomposition of aesculin tests according to Kageyama et al. (2004).

**Susceptibility testing.** Nocardia isolates were tested for antimicrobial susceptibility by Etest (AB Biodisk) in Mueller–Hinton blood agar (REDI). Strips used were ceftriaxone, imipenem, ciprofloxacin, co-trimoxazole, gentamicin, tobramycin, amikacin, linezolid and minocycline. All plates were incubated at 37 °C for 48 h. The MICs were interpreted in accordance with the National Committee for Clinical Laboratory Standards guidelines and those provided by the manufacturer (AB Biodisk).

**Clinical presentation.** Clinical data retrieved included: age, active comorbidities, immunosuppressive therapy, corticosteroid therapy, diabetes mellitus, chronic obstructive pulmonary disease (COPD), lymphoreticular neoplasm, organ transplantation, and human immunodeficiency virus (HIV) infection that was staged according to CDC classification (Centers for Disease Control and Prevention, 1992). The classification of comorbidity conditions, which can alter the risk of mortality, was studied by means of the Charlson comorbidity index (Sundararajan et al., 2004). The main outcome measure was crude mortality. Other outcome measures were attributable mortality, intensive care unit admission, length of hospital stay and readmission.

Nocardia infection was diagnosed when a patient developed consistent clinical symptoms together with isolation of a Nocardia species from a clinical specimen. Disseminated nocardiosis was defined as the isolation of Nocardia from two or more non-contiguous organs.

Statistical analysis was performed using an SPSS statistics package; differences between groups were analysed with the $\chi^2$ test or Fisher’s exact test. The significance level was set at 0.05 (two-sided).

**RESULTS AND DISCUSSION**

The taxonomy within the genus Nocardia is changing rapidly as the recognition and description of new species continues (Brown-Elliott et al., 2006; Saubolle & Sussland, 2003). Current molecular taxonomy provides an update on the taxonomy of the Nocardia spp. and points out many changes. In our study a total of 27 patients with documented nocardiosis were diagnosed from 1997 to 2003. Among them, eight different Nocardia species were isolated. Nine of the twenty-seven isolates (33.3 %) were identified as Nocardia farcinica. The isolation of N. farcinica is highly variable among countries. It has been reported to constitute 23.8 % Nocardia isolates in France (Boiron et al., 1992), 26.7 % in Japan (Kageyama et al., 2004), 35.4 % in Thailand (Poonwan et al., 2005), 44 % in Belgium (Glupczynski et al., 2006; Wauters et al., 2005) and 60.3 % in Germany (Schaal & Lee, 1992). Six of the twenty-seven isolates (22 %) were identified as N. abscessus. Infections by N. abscessus have only been reported in five patients in Japan (Kageyama et al., 2004), in one German patient who developed a mycetoma after a road accident (Horré et al., 2002) and, recently, in a disseminated infection in an HIV-infected Argentinian patient (Diego et al., 2005). Six isolates were identified as Nocardia cyriaci-georgica (22 %). Nocardiosis due to this species has recently been reported (Yassin et al., 2001) and it represents 10.2 % of the Nocardia isolates in a Japanese study (Kageyama et al., 2004). Two isolates were identified as N. otitidisca- viarum (7 %), and one each as Nocardia carneae, Nocardia nova and Nocardia transvalensis complex. The N. transvalensis complex isolated showed a 99.59 % 16S rDNA gene region homology with N. transvalensis (GenBank accession no. AY262304) and 99.58 % with N. asteroides ATCC 49872. Finally, one isolate was identified as a member of the N. nova complex; this isolate showed a 99.78 % 16S rDNA gene region homology with N. nova ATCC 33727 and 99.78 % with Nocardia veterana DSM 44445.

Patients’ demographic and clinical data are summarized in Table 1. A total of 19 patients (70 %) were male, 18 (67 %) were over 65 years and the mean age was 69 years. Mean hospitalization time was 20 days. Three patients (11.1 %) required intensive care unit admission and two patients with cellular immunosuppression (one patient with Hodgkin lymphoma and one on chronic corticosteroid therapy for advanced COPD) required readmission for Nocardia infection. All patients had active comorbidities at the time of infection. Charlson comorbidity index ranged from 0 to 7, with a mean of 2.8. Charlson index scores over 4 were obtained in six patients (22 %), N. farcinica was isolated from four of these and N. abscessus from two. Nineteen patients had COPD, three were diabetic, and two had solid neoplasms. Furthermore, 19 were immunosuppressed due to chronic corticosteroid therapy ($n=15$), immunosuppressive therapy ($n=6$), haematological malignancies ($n=3$) or HIV infection ($n=3$). All HIV-infected patients died, and two of the three were classified as stage C3 with disseminated nocardiosis caused by N. farcinica. Nocardiosis was pulmonary in 21 of the 27 patients (77.7 %), disseminated in 5 (18 %) and cutaneous in 1 (3.7 %). Four out of the five patients with disseminated disease had pulmonary involvement, two cutaneous dissemination and one cerebral disease. It is well known that pulmonary and disseminated nocardial disease is observed in immunocompromised patients, whereas primary cuta-
neous nocardiosis is usually an infection of immunocompetent hosts (McNeil et al., 1995).

The association between nocardiosis and underlying diseases has been well documented (Boiron et al., 1992; Lerner, 1996; Saubolle & Sussland, 2003; Sorrell & Mitchell, 2000). In our study no significant differences in age or comorbidity parameters were found between patients with the different species of Nocardia. Outcome was only known in 26 of the 27 patients. Therefore, the crude mortality rate was 50 % (13/26 patients), ranging from 38.1 % in localized nocardiosis to 100 % in disseminated disease. Attributable mortality was 34.6 % (9/26 patients). Reported rates of crude mortality ranged from 29 to 85 % and attributable mortality from 10 to 26 % (Jones et al., 2000; Pintado et al., 2002; Sánchez Muñoz-Torrego et al., 1995; Santos et al., 2002).

The slow growth of Nocardia species is a major difficulty for susceptibility testing (Tomlin et al., 2001), and in our

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**Table 1. Clinical features, therapy and outcome of patients with nocardiosis**

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<th>Age/sex</th>
<th>Species</th>
<th>Charlson index</th>
<th>Active comorbidity</th>
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*Isolates were susceptible to all regimens used in the treatment, except for the isolate from the patient who was treated with levofloxacin.*

F, Female; M, male; HCV, hepatitis C virus; SLE, systemic lupus erythematosus; N, no; Y, yes; D, died; S, survived.
experience the results are not easily interpreted when different methods are compared. *Nocardia* species can vary in their antimicrobial susceptibility patterns (Saubolle & Sussland, 2003). In agreement with previous results (Conville et al., 2003; Pottumarthy et al., 2003), in our series of tests antimicrobial agents such as co-trimoxazole (MICs 0.012–0.75 μg ml⁻¹), amikacin, linezolid and minocycline appeared to be highly effective in vitro against the eight *Nocardia* species isolated. Imipenem resistance (MIC ≥ 32 μg ml⁻¹) was observed in six *N. abscessus*, two *N. otitidiscaviarium* and one out of six *N. cyriacigeorgica* isolates. Although *N. abscessus* isolates were resistant to imipenem they were susceptible to ceftriaxone (MIC range 0.016–0.25 μg ml⁻¹). Only *N. otitidiscaviarium* was resistant to ceftriaxone. *N. nova* complex, *N. transvalensis*, three out of six isolates of *N. abscessus* and four out of six isolates of *N. cyriacigeorgica* were resistant to ciprofloxacin. All *N. farcinica* isolates and *N. nova* complex were resistant to both gentamicin and tobramycin, and susceptible to amikacin. In contrast, *N. cyriacigeorgica*, *N. nova*, *N. abscessus* and *N. otitidiscaviarium* were sensitive to all aminoglycosides tested. *N. nova* complex and three isolates of *N. cyriacigeorgica* were minocycline-resistant. *N. carnea* was susceptible to all antimicrobials tested (Table 2). The patterns of susceptibility to β-lactam agents, ciprofloxacin, gentamicin and tobramycin in our isolates varied among the species, as in other publications. (Ambaye et al., 1997; Brown-Elliott et al., 2001)

A total of 23 patients were treated with antibiotics: 14 (51.9 %) received co-trimoxazole, 4 (14.8 %) sulfadiazine, 2 (7.4 %) cephalosporins, 1 imipenem plus an aminoglycoside, 1 amoxiclav and 1 levofloxacin. In our series of tests, different treatments did not appear to be related to patient outcome. Isolates from 22 of the 23 patients who received antibiotic treatment were susceptible by Etest to the assigned treatment. One patient received levofloxacin, and although the strain was resistant to ciprofloxacin in vitro, the patient had a favourable outcome. Clinical outcome does not always correlate with laboratory susceptibility (Bani-Sadr et al., 1995; Threlkeld & Hooper, 1997).

Clinical experience and in vitro antibiotic-susceptibility analysis have shown that management of *Nocardia* infections must be individualized. The treatment of choice for this infection is the trimethoprim–sulfamethoxazole combination (Lerner, 1996), despite the controversy over its synergistic effect in vivo (Lerner, 1996; Moylett et al., 2003). Nevertheless, treatment failures have been described when using it alone, especially in disseminated and central nervous system nocardiosis. These specific situations may require faster primary bactericidal agents such as imipenem, amikacin, minocycline, linezolid or cephalosporins (Lerner, 1996; Saubolle & Sussland, 2003; Threlkeld & Hooper, 1997). Moreover, some authors recommend various multi-drug regimens for the management of these disseminated infections (Betriu, 1997; Lerner, 1996; Saubolle & Sussland, 2003; Threlkeld & Hooper, 1997). Optimal regimens for disseminated or central nervous system nocardiosis could therefore be a combined therapy including a sulfonamide and a bactericidal primary agent.
or a combination of imipenem and amikacin. Clinical and in vitro effectiveness for other combined regimens, including wide-spectrum cephalosporins, amoxicillin-clavulanate and fluoroquinolones, has also been reported (Saubolle & Sussland, 2003; Sorrell & Mitchell, 2000; Threlkeld & Hooper, 1997).

In summary, nocardiosis may be a localized or a disseminated infection, which manifests clinical and microbiological differences depending on the infecting Nocardia species. Our study showed the prevalence of the eight different Nocardia species during a period of 7 years. N. farcinica infections were seen in patients with a higher comorbidity, were more often disseminated and had a higher mortality rate. Furthermore, N. farcinica isolates were more resistant in vitro. N. abscessus is the second most prevalent species in our study. Accurate identification to the species level and susceptibility testing of Nocardia isolates may thus be important in patients’ diagnosis and treatment.

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REFERENCES


